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VOL. 20, SEC. C. JUNE, 1942 NUMBER 6

THREE STRAINS OF CUCUMBER MOSAIC OCCURRING ON TOBACCO IN ONTARIO AND QUEBEC[1]

BY J. H. H. PHILLIPS[2]

Abstract

Cucumber mosaic was found affecting tobacco plantings in both Ontario and Quebec. From diseased material collected in these regions three strains were isolated and designated as Strains 1, 2, and 3. Strain 1 most closely resembled typical cucumber mosaic in its symptoms on tobacco and tomato. Strain 3 produced a similar type of mottle to Strain 1, but was generally more severe and consistently produced severe leaf narrowing on tomato. Strain 2 was easily recognized by its ability to produce necrotic rings on the inoculated leaves of burley tobacco varieties and the tendency of affected plants to recover from the initial symptoms. The three strains retained their identity through a large number of serial inoculations.

Investigations demonstrated that a severe type of streak was produced when tomato plants were inoculated with a combination of cucumber mosaic virus (Strain 3) and potato X virus.

Unlike tobacco mosaic virus, the virus of cucumber mosaic was unable to survive over winter in plant tissue in the soil. Field observations indicated that dissemination of cucumber mosaic in tobacco plantings was effected by insect vectors.

Since Johnson (6) first described cucumber mosaic on tobacco, it has been recognized as being widely distributed on this host. Valleau and Johnson (10) in 1928 reported it from Kentucky as "Puffed" disease, and Johnson (5) in 1930 described three strains occurring on tobacco in Kentucky. Several strains have since been described by various workers and the symptoms and properties of the virus have been extensively studied.

In 1939, considerable cucumber mosaic was observed at St. Catharines in tobacco plots in which tobacco mosaic was being studied. At that time the symptoms, especially in the early stages of infection, were easily confused with those of tobacco mosaic and the identity of the virus concerned was often ascertained only by inoculation to other hosts. There was also considerable variation in the symptoms produced, which suggested the possibility of more than one strain of cucumber mosaic being involved. For this reason a study of the symptoms of cucumber mosaic as it occurs in Ontario was considered advisable.

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Contribution No. 694 from the Division of Botany, Science Service, Department of Agriculture, Ottawa, Canada.

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Distribution and Importance

Tobacco leaves showing symptoms of cucumber mosaic have been received at St. Catharines from the tobacco growing areas of both Ontario and Quebec. Though cucumber mosaic occurs in both the old and new tobacco belts in Ontario, it does not appear to be widespread or to cause much damage to the crop. In Quebec, however, where tobacco is grown in comparatively small plots often adjacent to vegetable crops such as potatoes, cucumbers, tomatoes, etc., cucumber mosaic on tobacco is more prevalent.

Investigations at St. Catharines indicate that certain strains of the cucumber mosaic virus are capable of causing severe injury to tobacco, while other strains cause little or no injury beyond mild stunting. Because of its superficial resemblance to tobacco mosaic, many growers in Ontario and Quebec are not aware of the occurrence of this disease on tobacco.

Material and Methods

Tobacco and weed hosts infected with cucumber mosaic were collected from both Ontario and Quebec. Of the many strains 'isolated' from this material, three gave consistent differences on a certain host range and were used for further study. Some 2000 plants including 18 varieties of tobacco and 16 other hosts were inoculated during the course of these investigations.

Inoculations were made by the leaf rubbing method. Infected tissue was macerated in a sterile mortar, diluted with one part of tap water and gently rubbed over the leaf of the healthy plant with a pad of sterile gauze. Juice for thermal inactivation tests was prepared in a similar manner and then centrifuged for two to three minutes. It was then poured into 3 cc. stoppered glass tubes and plunged into a constant temperature water-bath for the desired period.

Plants were grown in 5-in. pots and usually were inoculated as soon as two or three large leaves had developed. They were kept under daily observation and symptoms were recorded as they appeared. All plants were grown in screened greenhouse compartments which were fumigated regularly to control insects. With one or two exceptions all experiments were repeated several times and doubtful transmissions of the virus were checked by inoculations back to tobacco.

Symptoms of the Three Strains

Under greenhouse conditions, the symptoms produced by the three strains on cucumber var. Chicago Pickling were very similar and resembled those described for cucumber mosaic by Doolittle (3) except that no mottling was observed on the fruit. When small cucumber plants were inoculated, they usually died before setting fruit. Any fruits that were produced were small and misshapen with undeveloped narrowed tips. Plants inoculated in the field showed similar symptoms, though the plants were not killed, a small rosette of leaves remaining alive at the tips of the vines.

The three strains were differentiated, however, by the symptoms they produced on tobacco and tomato, as shown in Table I.

TABLE I

DIFFERENTIATION OF CUCUMBER MOSAIC STRAINS ON TOBACCO AND TOMATO

Host	Strain 1	Strain 2	Strain 3
Tobacco var. Harrow Velvet	(i) Large, diffused primary pale green lesions (ii) Vein clearing and paling of young leaves (iii) Blotchy pale green mottle, leaf tips narrowed and chlorotic, some stunting of plants	(i) Large primary yellow lesions with concentric necrotic rings (Fig. 1) (ii) Vein clearing and general paling or vein-banding of young leaves with fine necrotic spotting (iii) Fine mosaic mottle often with definite ring spotting, plants tend to recover from the initial symptoms but are paler than normal	(i) Large, diffused primary yellow lesions, may become necrotic (ii) Vein clearing and paling of young leaves (iii) Blotchy yellow mottle, leaves narrowed with elongated, chlorotic tips, some leaf distortion and blistering, severe stunting
Tomato var. Grand Rapids	(i) Primary pale green lesions (ii) Vein clearing and paling of young leaves (iii) Coarse blotchy pale green mottle, leaf narrowing usually slight, some stunting	(i) Necrotic local lesions (ii) Vein clearing of young leaves (iii) Plants may be paler than normal, no mottle	(i) Large diffuse primary yellow lesions (ii) Paling or yellowing of young leaves (iii) Severe leaf narrowing, 'fern leaf'. Plants partially recover from severe leaf narrowing, have yellow mottle, severe stunting

All of the following hosts were also successfully inoculated with each of the three strains:— *Nicotiana glutinosa* L., *N. rustica* L., *N. Langsdorffii* Wunn., *N. sylvestris* Spegaz & Comes., *N. glauca* Grah., *N. Bigelovii* S. Wats., *Physalis angulata* L., *Capsicum frutescens* L., *Nicandra physalodes* (L.) Pers., *Phytolacca decandra* L., *Antirrhinum* sp., *Datura Stramonium* L., *Zinnia* sp., and *Petunia* sp.

Strains 1 and 3 generally produced a coarse, blotchy mottle of a similar type on all of these hosts with or without primary yellow lesions. Strain 3, however, could usually be distinguished by its more contrasting mottle and a tendency to cause severe stunting. On *Datura Stramonium* only a mild ring spot type of mottle was produced by either of these two strains. This tended to fade and affected plants later appeared almost normal. Strain 2 consistently produced a mild mottle of a finer type than Strains 1 and 3 without the leaf narrowing or severe stunting. On the following hosts this mottle was accompanied by a fine necrotic or ring spotting similar to that produced on tobacco:—*N. rustica, N. glutinosa, P. angulata, N. physalodes.* On all the

other hosts a systemic mild mottle only was produced and plants often tended to recover and produce normal growth.

Under conditions of poor light and poor plant growth, transmission of cucumber mosaic to tobacco by mechanical injury was often difficult. Symptoms were slow in appearing and showed considerable variation in severity. Primary yellow lesions were often not visible until after systemic symptoms had appeared, and inoculated leaves tended to become necrotic. Under winter conditions the symptoms on tomato produced by Strain 1 may approach the 'fern leaf' symptoms of Strain 3, but under more favourable growing conditions Strain 1 produced only a coarse mottle, whereas Strain 3 produced the severe leaf narrowing symptoms. Attempts to duplicate these symptoms in the field failed, since considerable difficulty was experienced in inoculating tomato plants and there was a high incidence of natural infection by both cucumber mosaic and tomato mosaic viruses.

Under field conditions, the symptoms of cucumber mosaic on tobacco corresponded to those produced by the three strains in the greenhouse. Strain 2 could be distinguished readily from Strains 1 and 3 by the large, distinct necrotic rings on the inoculated leaves, the necrotic flecking and ring spotting on the leaves which showed the primary systemic symptoms and the tendency of the plants to recover from the mottle. Though Strains 3 and 1 produced a similar type of coarse mottling, Strain 3 induced a more severe type of breakdown on inoculated leaves and caused more pronounced narrowing of leaves and more severe stunting of plants. The three strains all tended to produce a necrotic breakdown of the older leaves, especially along the veins, in the form of elongated necrotic spots, line patterns, and ring spots. It was interesting to note that in the field burley varieties developed more severe symptoms and a more contrasting mottle than flue-cured varieties. This was especially noticeable with Strain 3 which produced a distinct yellow mottle on the burley varieties and a much less contrasting pale green mottle on the flue-cured varieties.

In comparison with tobacco mosaic the symptoms of cucumber mosaic on tobacco, though usually less severe, were nevertheless more chlorotic. Unlike tobacco mosaic the young leaves at the top of a plant infected with cucumber mosaic, after the initial systemic symptoms, appeared almost normal with only scattered pale green or yellow areas or a mild mottle. As these leaves expanded they developed the characteristic blotchy mottle or general paling characteristic of the strain. In the early stages of infection on tobacco it was often difficult to distinguish cucumber mosaic from tobacco mosaic in that they both produced a vein clearing, and often a pale green to yellow vein banding of the young leaves. In the later stages of infection, however, plants infected with tobacco mosaic virus showed a uniform mottling of the leaves with blistering and distortion, whereas those infected with cucumber mosaic virus showed a coarse, blotchy mottle with only occasional small blisters, but with the leaf tips chlorotic and narrowed.

PLATE I

FIG. 1. *Strain 2 showing the necrotic rings on the inoculated leaves and the paling and necrotic flecking of young leaves of Harrow Velvet.*

Cucumber Mosaic in Relation to Tomato Streak

During the course of the investigation cucumber mosaic was also recovered from greenhouse tomato plants showing typical streak symptoms. The possibility that cucumber mosaic may be involved in the production of a 'streak' of tomatoes was investigated.

Tomato plants in the greenhouse were inoculated with one of the three strains of cucumber mosaic, together with one of the following: (*a*) *Nicotiana* Virus 1 (9)—ordinary tobacco mosaic which produced primary yellow lesions and a systemic mosaic mottle on tomato; (*b*) *Lycopersicum* Virus 1—a mild strain of tomato streak virus which produced necrotic local lesions on tobacco var. Yellow Mammoth but only a systemic mosaic mottle on tomato; (*c*) *Solanum* Virus 1 (potato X virus)—a strain recovered from potato, which produced a rather severe mottle on tomato with some necrotic ring spotting as the initial symptoms, but from which plants tended to recover.

It was found that Strain 1 was almost entirely masked by each of the three viruses with which it was inoculated. Strain 2 tended to produce necrotic local lesions when inoculated along with either tomato streak virus or potato X virus and increased the amount of necrotic spotting in a potato X virus combination. Strain 3 inoculated along with either tobacco mosaic virus or tomato streak virus produced a necrosis on tomato of a relatively mild type as compared to the usual 'combination streak' encountered. With potato X virus, however, Strain 3 produced streak symptoms of a severe type closely resembling those produced by potato X virus plus tobacco mosaic virus.

Properties

All three strains failed to survive heating to 65° C. for 10 min. and failed to withstand drying in the leaf tissue. Great difficulty was experienced in recovering the virus from leaves dried for more than 48 hr. at room temperature.

Overwintering

Doolittle and Walker (4) have shown that the virus of cucumber mosaic overwinters in perennial hosts or in the seed of wild cucumber and does not persist in the soil for any length of time. On the other hand Johnson (7) in Wisconsin and Berkeley (2) in Ontario have shown that the virus of tobacco mosaic is capable of wintering over in the soil and infecting tobacco, if tobacco follows tobacco in the rotation. However, since cucumber mosaic is often present in tobacco plantations in Ontario, it was considered advisable to test for the possibility of its overwintering in the soil under Ontario conditions.

Accordingly, in the spring of 1940 all plants in a plot of 200 tobacco plants (plot No. 1) were inoculated with cucumber mosaic and in October of the same year the inoculated plants were cut up and incorporated with the soil. A similar plot was inoculated with tobacco mosaic virus (plot No. 2) and treated in a similar manner. These plots were cultivated only when the plants were very small and every care was taken to avoid contamination from outside

sources. A check plot of 200 uninoculated plants (plot No. 3) treated similarly remained free from either cucumber or tobacco mosaic throughout the season. At intervals throughout the winter samples of soil from these plots were brought into the greenhouse and tested for the presence of virus by rubbing gently the leaves of *N. glutinosa* with a soil suspension of these samples which had been previously stirred in a sterile mortar, in order to extract any virus present in the soil. The remaining soil was put into 5-in. pots and planted with young, vigorously growing tobacco plants. The results of these tests are shown in Table II.

TABLE II

THE RESULTS OF TESTS MADE DURING THE WINTER WITH SOIL FROM PLOTS IN WHICH CUCUMBER MOSAIC AND TOBACCO MOSAIC PLANTS HAD BEEN INCORPORATED WITH THE SOIL

Date of taking sample	Soil from tobacco mosaic plot No. 2		Soil from cucumber mosaic plot No. 1	
	Soil suspension inoculation	Plants potted in soil	Soil suspension inoculation	Plants potted in soil
Dec. 3, 1940	3/3*	4/3	3/0	4/0
Jan. 3, 1941	4/4	4/2	4/0	4/0
Feb. 1, 1941	4/4	4/2	4/0	4/0
Mar. 31, 1941	4/4	4/0	4/0	–
May 17, 1941	4/3	4/0	–	–

* *Upper figure indicates number of plants inoculated or planted, lower figure indicates number of plants infected one month from the date of inoculation.*

The results recorded in Table II would suggest that the cucumber mosaic virus did not persist in the soil during the winter of 1940, whereas the tobacco mosaic virus did overwinter in the soil.

In the spring of 1941 when these plots were replanted to tobacco, cucumber mosaic appeared within one month of planting, but it was not significantly more prevalent in plot No. 1 than in plot No. 2 and in No. 3. On the other hand, plot No. 2 showed 4% infection with tobacco mosaic one month after replanting, but only one plant infected with tobacco mosaic appeared in plot No. 1, thus indicating that tobacco mosaic had overwintered in the soil. The uniformity of infection with cucumber mosaic in both the inoculated and non-inoculated plots in the spring of 1941 would further indicate that in plot No. 1 the soil was not the source of infection for these plants. It is more probable that perennial weeds adjacent to the plots served to carry over the virus.

In a series of plots for the study of other viruses it was observed that cucumber mosaic spreads much more rapidly than tobacco mosaic and that the location of infected plants could not be correlated with the soil or cultural practices. Rather the observations suggested that infection was more likely to be due to insect vectors, especially since the aphid, *Macrosiphum gei* Koch, occurs quite commonly on tobacco at St. Catharines and in limited tests readily transmitted cucumber mosaic to this host.

Discussion

The results of these investigations of cucumber mosaic on tobacco in Ontario are for the most part in agreement with those of workers in other parts of the world. Though only three distinct strains were encountered in Ontario and Quebec, more might have been obtained if the selective method used by Price (8) in his separation of yellow strains had been employed. The strains recorded here were selected because they were of common natural occurrence. They gave a consistent symptom picture throughout a large number of transfers and were quite distinct and identifiable in the field in comparable infections. The identification of these strains with those described by previous workers presents difficulties but in general Strain 1 resembles the cucumber mosaic of Doolittle (3) in America and the yellow mottle mosaic of cucumber of Ainsworth (1) in England.

Strain 3 appears to be similar in its action on tomato to the Strain 3 used by Valleau and Johnson (11) in their experiments with 'streak' of tomato. Strain 2 may be a new strain, as it could not be identified with any strain encountered in the literature. It is not suggested that these are pure strains, as they may be relatively stable combinations of more than one strain. Their reaction on cucumber, their thermal death point and host range do, however, tend to confirm that they are all strains of *Cucumis* Virus 1 (9).

The apparently limited occurrence of cucumber mosaic in tobacco plantings in Ontario may be due to it being confused with tobacco mosaic. At certain stages of infection the symptoms may be very similar but when the typical mottling is present, they are easily distinguished.

Unlike tobacco mosaic which persists over winter in the soil and is spread mainly by contact, cucumber mosaic apparently is spread by insects from overwintering perennial hosts. It is therefore apparent that control measures as applied to tobacco mosaic would not be effective in controlling outbreaks of cucumber mosaic.

Acknowledgment

The author wishes to express his appreciation to Dr. G. H. Berkeley for suggesting and directing this investigation and for his guidance in the preparation of this paper.

References

1. Ainsworth, G. C. Ann. Applied Biol. 22(1) : 55-67. 1935.
2. Berkeley, G. H. Can. Dept. Agr. Circ. 163. 1940.
3. Doolittle, S. P. U.S. Dept. Agr. Dept. Circ. 321. 1924.
4. Doolittle, S. P. and Walker, M. N. J. Agr. Research, 31(1) : 1-58. 1925.
5. Johnson, E. M. Kentucky Agr. Expt. Sta. Bull. 306. 1930.
6. Johnson, J. Phytopathology, 16(2) : 141-149. 1926.
7. Johnson, J. J. Agr. Research, 54(4) : 239-273. 1937.
8. Price, W. C. Phytopathology, 24(7) : 743-761. 1934.
9. Smith, K. M. A textbook of plant virus diseases. J. & A. Churchill, Ltd., London. 1937.
10. Valleau, W. D. and Johnson, E. M. Abstract. Phytopathology, 18(1) : 132-133. 1928.
11. Valleau, W. D. and Johnson, E. M. Phytopathology, 20(10) : 831-839. 1930.

THE BEHAVIOUR OF RESAZURIN IN MILK[1]

By C. K. Johns[2]

Abstract

The view that resazurin is not a satisfactory substitute for methylene blue in assessing the hygienic quality of milk has been examined.

Resazurin is equally as sensitive as methylene blue to the metabolic activities of bacteria in milk. It is decidedly more sensitive to the presence of non-bacterial factors in abnormal milks (mastitis, late lactation, etc.) and therefore furnishes a more comprehensive index of the true quality of the milk.

Potentiometric studies with a wide variety of milks have failed to support the view that resazurin exerts such a strong poising action in market milks as to complicate the test or interfere with the interpretation of results. No evidence was obtained in support of the claim that differences in poising properties of different milks are of sufficient magnitude to affect the results of the test.

The suitability of the dye resazurin for determining the hygienic quality of milk has been questioned seriously by Thornton, McClure, and Sandin (29). These workers state that "a significant proportion of results cannot be interpreted with the reasonable certainty necessary to justify the widespread use of these (resazurin) tests by the dairy industry". Since this view runs counter to those of various other investigators (2, 3, 4, 15, 18, 19, 20, 21, 22, 23), it seemed desirable to examine in some detail the objections raised by the aforementioned workers.

In the paper referred to, two points in particular have been stressed. The first is that the degree of colour change in resazurin–milk mixture does not always correlate satisfactorily with the bacterial content as indicated by the methylene blue reduction test or other method. This springs from the fact, reported by various workers (2, 7, 16, 18, 19, 22, 23, 24, 26, 27), that resazurin is much more sensitive to the presence of non-bacterial factors (leucocytes or substances accompanying them) than is methylene blue. Consequently, milk from diseased or otherwise abnormal udders will show a significant degree of colour change in resazurin during the first few hours of incubation, even though the bacterial content is low. Resazurin is therefore much more useful than methylene blue in detecting such abnormal milks, while equally as valuable in reflecting excessive bacterial numbers.

The influence of these non-bacterial factors, which appear to be responsible for discrepancies between the results of the resazurin "one hour" (23) or "triple reading" (18) tests and the methylene blue reduction test, has been generally recognized by other workers. Thornton *et al.* appear to ignore this influence, preferring to judge the test exclusively on its ability to indicate bacterial numbers. The desirability of a broader concept of milk quality has previously

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been discussed (16, 18). It may also be pointed out that these non-bacterial factors have little influence upon the time required for resazurin to reach the full pink stage (colour No. 16) in market milks (18), as indicated by the close correlation between reduction times of resazurin to pink and methylene blue to white (3, 15, 17). Consequently, if the hygienic quality of milk is to be judged solely by the bacterial content, the resazurin "pink" test may be substituted for the methylene blue test with an appreciable shortening of the reduction time (3, 15).

The second point stressed by Thornton *et al.* is the strong poising action of resazurin in milk. Such statements as "milk was immediately overpoised by the resazurin", "the possibilities of overpoising effects", etc., appear throughout their paper. Poising action refers to resistance to change in potential upon the addition of an oxidizing or reducing agent. Two factors are concerned; (*a*) the intensity, represented by the E_o value which characterizes a *reversible* oxidation–reduction system, and (*b*) the capacity or concentration factor. Neither the E_o values nor the concentrations of the systems responsible for the initial potential level of milk are known. Ramsdell *et al.* (23) stated that resazurin is slightly more electropositive than methylene blue but gives no E_o value; Thornton *et al.* reported obtaining a perfect two-step titration curve at approximately pH 1, but failed to present their curve or the E_o value for resazurin. Almost the only known factor in a resazurin–milk mixture is the concentration of the dye. Assuming the dye to be 100% pure[1], the customary strength in milk (1 in 200,000 parts) represents a concentration of 0.000022 *M*. It seems most unlikely that this low concentration of an irreversibly[2] reducing dye system could immediately overcome the initial potential of the milk and force it to a significantly lower level. (This is presumably what Thornton *et al.* mean by the term "overpoising".) The previously published data on the influence of various concentrations of resazurin upon the time–potential curve show that even double the usual concentration of the dye fails to change the potential level of the milk until bacterial activity enters into the picture (17). The only evidence of any poising action by the dye manifests itself when the resazurin has been almost completely reduced to the pink component, resorufin. Since the reduction of resorufin to the colourless compound is reversible, poising action at this stage is not unexpected. The shorter time required to reach the pink stage, compared with the decolorization of methylene blue, was reported (17) as being mainly attributable to the change in shape of the time–potential curve owing to the poising action in the vicinity of the pink stage.

The view of Thornton *et al.* that resazurin is of dubious value *because it tends to force the potential of milk downward to a lower level* appears to be based mainly upon the few time–potential curves presented in their paper.

[1] *By the titanous chloride method, the lot of resazurin used here was found to be 95% pure (Eastman Kodak Company, April 12, 1939).*

[2] *While the change from pink to white is reversible, the previous change from initial blue (resazurin) to pink (resorufin) is irreversible in milk (23, 29).*

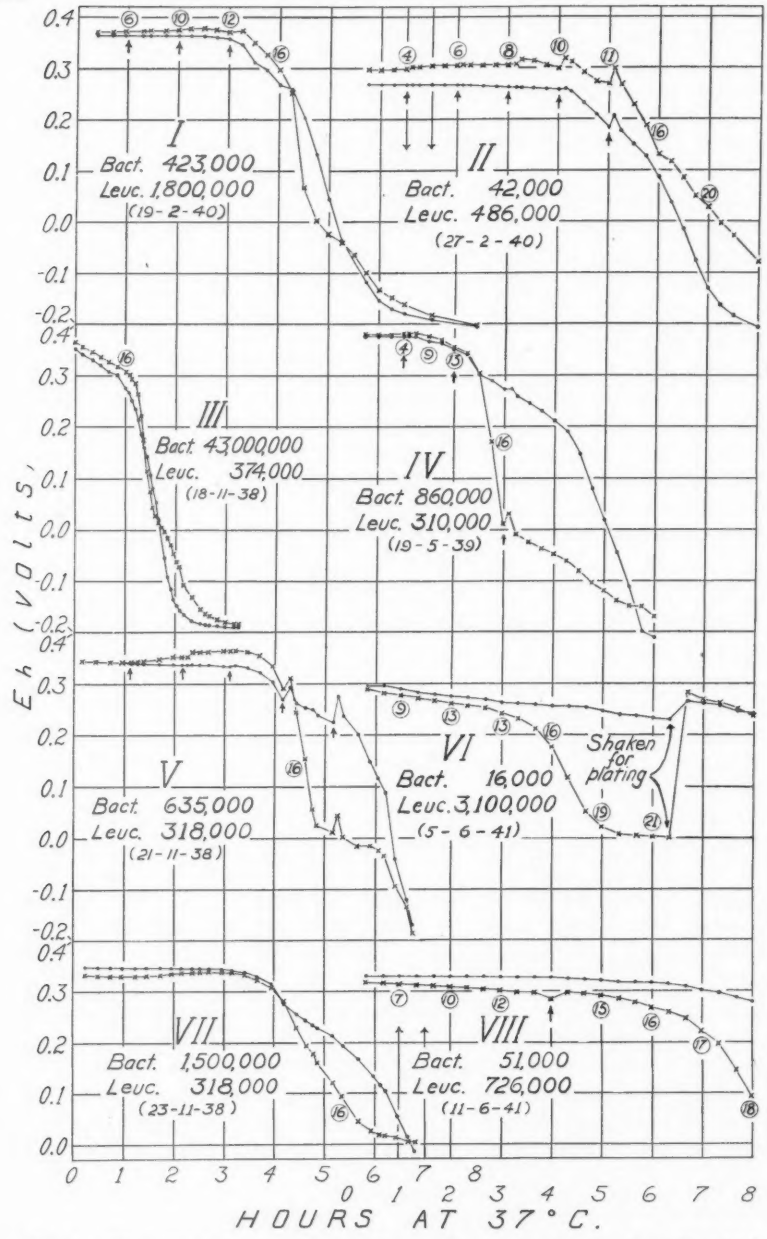

FIG. 1. *Time–potential curves of milks with and without resazurin. Nos. I and II = herd milks, Nos. III, IV, V, and VII = vat milks, Nos. VI and VIII = milks from individual cows. Counts made by direct microscopic method, counting 60 fields. Arrows indicate inversion of tubes to redistribute cream layer. Ringed numbers represent resazurin colours (0 = initial, 16 = pink, 24 = discolorized).* ×———× *= resazurin;* •———• *= no dye.*

With the exception of Milk 2 in their Fig. 1, these curves differ significantly from those previously published (17). A further series of representative curves is shown in Fig. 1. It will be noted that even with Milks VI and VIII from cows with mastitis, the curve for milk + resazurin closely parallels that for plain milk for several hours. In no instance is there any suggestion that resazurin causes downward displacement of the potential level of the milk during the first few hours. Even in Milk III with an extremely high bacterial content and short reduction time, there is no hint of the "overpoising effect" mentioned by Thornton *et al.*

Two possible explanations for these differences in findings suggest themselves: (1) some inherent difference in the behaviour of their milks and of ours; and (2) differences in the methods employed in measuring potentials.

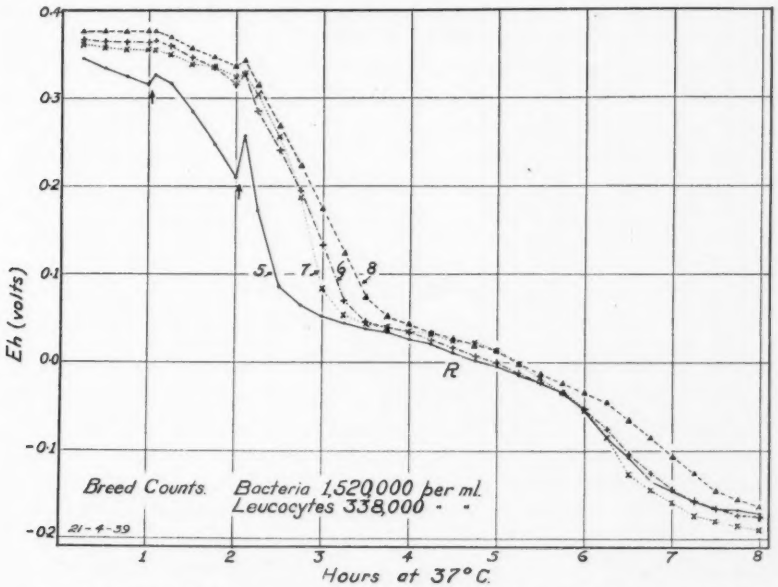

FIG. 2. *Time–potential curves for four electrodes in milk + methylene blue. Tubes inverted at points marked ↑. Dye reduced at Point R.*

We know of no evidence that milks in the Edmonton district behave so differently from those in the Ottawa area that this would account for the above-mentioned differences in the shapes of time–potential curves. On the other hand, it is generally recognized that a single electrode may give aberrant readings upon occasion, even though at other times it behaves normally. The curve for electrode No. 5 in Fig. 2 serves to illustrate this. In this instance the milk contained methylene blue thiocyanate (1 : 300,000). Again, a series of electrodes which have been found to be in close agreement when

checked against a standard solution of ferrous and ferric chloride may show a surprising degree of variation in initial potential level when inserted into different tubes containing portions of the same milk, as indicated by the data shown in Table I. The range between highest and lowest readings is 111 mv., more than the difference between the initial levels of the two curves for Milk 1 (their Fig. 1), which Thornton *et al.* state "was immediately overpoised by the resazurin".

TABLE I

DIFFERENCES IN INITIAL POTENTIALS AMONG 12 ELECTRODES IN SIX TUBES OF THE SAME MILK (JULY 24, 1941)

Tube No.	Electrode No.	Initial Eh, v.	Deviation from average, mv.
1	1	+0.274	−41
	2	+0.288	−27
2	3	+0.288	−27
	4	+0.331	+16
3	5	+0.385	+70
	6	+0.365	+50
4	7	+0.275	−40
	8	+0.305	−10
5	9	+0.309	− 6
	10	+0.323	+ 8
6	11	+0.317	+ 2
	12	+0.317	+ 2
Average		+0.315	
Extremes of variation			111

In view of the unreliability of readings from single electrodes, we have invariably used duplicate electrodes[1] in each tube, and, wherever possible, duplicate tubes. When one electrode appears to be behaving peculiarly, its readings are discarded. By thus obtaining two or more sets of readings on each sample, and by running a wide variety of samples, we feel that we are less likely to have been misled regarding the shape of the time–potential curves for milk containing resazurin or methylene blue.

Almost all workers in the field of biological oxidation–reduction recognize the need for avoiding errors resulting from polarization. As Wilson *et al.* (34) have expressed it, "Quite fictitious potential values can be obtained unless this tendency to polarization due to drawing of current through the electrode is avoided, particularly if the milk is unstirred, as is essential in reproducing the conditions of the reduction test". Polarization is difficult to avoid with the type of apparatus used by Thornton *et al.* (32). In view of the current

[1] *The electrodes and potentiometric set-up have been described in a previous paper (17).*

general use of apparatus which minimizes polarization, the statement of these workers that "Oxidation–reduction potentials were measured by means of the usual electrometric set-up . . . ." is likely to be misleading. Comparison of their curves with those obtained with more suitable apparatus (13, 16, 17, 34) suggests that polarization may indeed have influenced some of their readings. This, together with their reliance upon single electrodes, makes it difficult to dismiss the impression that the curves that they have published, and upon which they appear to base much of their objection to resazurin, are by no means truly representative.

The statement of Thornton *et al.* that "the time required to reach either the pink or white end-point was a function not only of bacterial influences but also of the comparative poising capacities of the milk and dye systems" suggests that differences in these poising properties may result in such marked discrepancies as to render the results of the resazurin test of questionable value. We have seen nothing to lead us to believe that such differences enter into the question. That such discrepancies rarely occur is also indicated by the close correlation between the resazurin (pink) and methylene blue reduction times (3, 15, 18, 19).

Discussing the possibility of shortening the reduction test by "overpoising" the milk, Thornton *et al.* state that "this appears feasible only if milks do not vary in their inherent reducing intensities and capacities". They believe "it is not unreasonable to assume varying poising in market milks" and that "interpretations of the behaviour in milk of such strongly poised systems as resazurin should be tempered with caution". No evidence is presented to substantiate this opinion, although reference is made to Greenbank's (12) work. Greenbank showed that when tested 6 to 48 hr. later, freshly drawn milks showed varying rises in Eh as a result of adding small amounts of copper. The difference between milks showing greatest and least change after six hours was 14 mv., which can scarcely be regarded as significant in relation to resazurin reduction. There was no indication that this had any bearing upon the point under discussion, i.e., that resazurin might "overpoise" certain milks. While we have seen nothing in our own studies to lead us to believe that variations in the poising properties of different milks are of sufficient magnitude to influence the results of the resazurin test, it seemed worth while investigating this point more fully. To date milks from 70 individual cows as well as 24 herd samples from different shippers have been studied.

We first attempted to show differences in initial potential and poising properties by measuring the change in potential 10 min. after the addition of 10 p.p.m. of copper, along the lines suggested by Fay *et al.* (6). (After the first few trials, however, we omitted adjustment of the pH of the milk to 6.35 as was done by these workers.) Changes in potential were very slight, the maximum noted being 12 mv.

We next studied the effect of added reducing agents, including cysteine, hydroquinone, sodium sulphite, and sodium thioglycollate. Fig. 3 depicts time–potential curves for six milks from individual cows showing varying

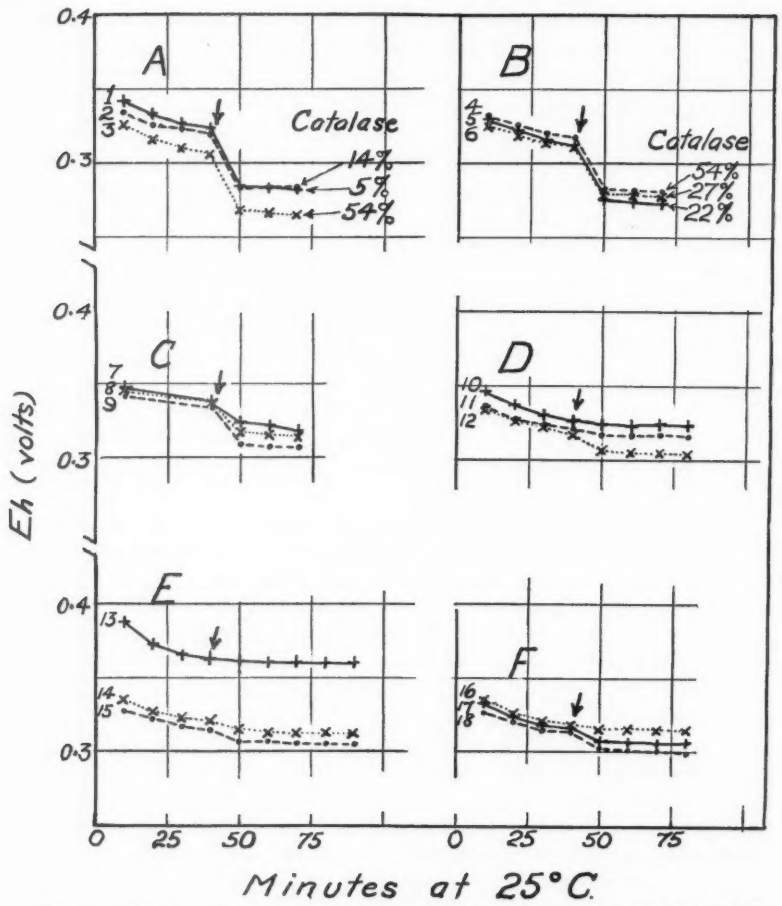

FIG. 3. *Effect upon Eh of addenda of various reducing agents. Samples 1 to 6 = individual cow milks, 7 to 18 = herd milks. A and B: 1 ml. 2.5% solution of hydroquinone added at ↓; C: 1 ml. 0.1% cysteine hydrochloride added at ↓; D, E, and F: 1 ml. 0.01% cysteine hydrochloride added at ↓.*

degrees of udder abnormality (*A* and *B*), as well as those for 12 herd milks (*C* to *F*). Each curvé represents the average of readings from four electrodes. These milks, representing about one-fifth of the total number studied, show such slight differences in poising properties that it seems most unlikely that the results of the resazurin test would be affected thereby. This contention is supported by the data in Fig. 4 showing the effect of repeated addenda of cysteine to milks with and without resazurin. In no instance has the presence of resazurin in the usual concentration caused any downward distortion of the time–potential curve. In fact, as has recently been reported (16), the presence

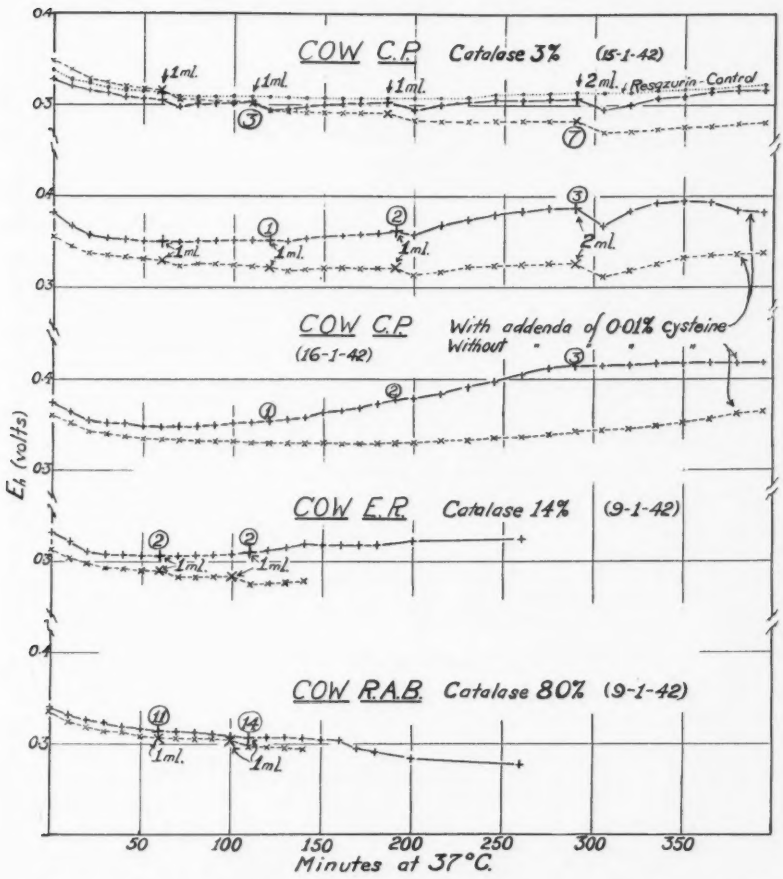

FIG. 4. *Effect of addenda of 0.01% solution of cysteine hydrochloride upon the Eh of milks with and without resazurin. Ringed numbers represent resazurin colours (0 = initial, 16 = pink).* +———+ *= resazurin added;* ×———× *= distilled water added.*

of resazurin in normal milk generally results in a slight *upward* trend in the curve. This tendency is absent from the milk of Cow *R.A.B.*, the abnormality of which is reflected in the much greater change in resazurin colour (as indicated by the ringed numbers) as well as in the slow downward trend of the curve.

Thornton *et al.* state "that some milks of low bacterial content, presumably abnormal, are rather strongly poised at Eh levels negative to the usual values of fresh milk". If this statement means that such milks show a lower initial level of Eh and a greater resistance to change of potential (stronger poising) than do normal milks, we are unable to agree. In Table II are shown Eh readings from a series of milks of varying degrees of abnormality.

TABLE II

Eh READINGS AND COLOUR CHANGES IN RESAZURIN–MILK MIXTURES INCUBATED AT 37° C. PAIL SAMPLES FROM COWS IN C.E.F. HERD. BACTERIAL COUNTS (PLATE) <10,000 PER ML.

Sample No.	Leucocytes	Catalase, %	Eh reading, mv.					Resazurin colour No. after:			
			Initial	1 hr.	2 hr.	3 hr.	4 hr.	1 hr.	2 hr.	3 hr.	4 hr.
1	3,700,000		359	360	365	370	375	6	9	9	10
2	3,310,000		285	271	272	272	257	9	10	11	12
3	3,100,000		288	279	263	239	176	9	13	13	16
4	2,000,000		329	323	306	280	182	11	13	17	19
5	1,590,000	44	308	298	286	275	260		10	13	14
6	1,500,000		366	365	364	365	365	4	8	8	9
7	1,270,000		370	368	366	359	340	8	11	13	13
8	1,160,000	48	311	314	314	315	316	7	11	12	13
9	970,000	58	312	314	322	329	337	6	9	10	11
10	890,000	33	332	328	329	331	334		9	13	14
11	860,000		287	278	277	272	272	2	7	8	9
12	830,000		314	313	304	300	292	7	10	12	12
13	740,000	25	326	333	343	355	356	2	5	6	8
14	726,000	26	315	311	304	299	283	7	10	12	13
15	500,000		297	301	309	320	326	3	7	7	8
16	413,000	50	357	354	355	357	361		8	10	11
17	391,000		305	307	317	327	337	2	4	6	6
18	381,000	15	338	341	343	343	348		5	8	8
19	381,000	31	285	280	279	282	288		9	12	15
20	359,000		315	316	315	316	312	4	6	7	10
21	342,000	19	294	299	284	286	293	3	5	8	9
22	300,000		339	339	344	348	356	6	7	10	10
23	211,000		313	314	317	322	327	4	6	6	6
24	159,000	14	358	353	349	352	349		4	7	7
25	123,000	7	318	317	320	324	328	3	4	4	6

These data fail to show any marked difference in initial Eh level correlated with leucocyte content or catalase value; on the other hand, they indicate, as do also the curves recently presented in another paper (16), that milks with high leucocyte content sometimes, but not invariably, show a slow downward drift of potential long before bacterial numbers are sufficient to account for the reduction. (See also curves for Milks VI and VIII, Fig. 1, and for Cow *R.A.B.*, Fig. 4.) We are unable to interpret these data as meaning that such milks are more strongly poised than normal milks.

The findings reported above, supplementing our experience with many hundreds of samples in the various modifications of the resazurin test, leave us with the conviction that differences in poising properties among different milks are too slight to have any significant influence upon the results of the test.

Significant differences in initial potential levels of milk have been reported by various workers. Thornton and Hastings (28) stated that "the positive Eh limits of all the milks examined lay between + 0.2 and + 0.3 volt. This corresponds closely to the limits reported by Clark and others for milk and some other biological fluids." Among others who have reported similar

potential levels are Fay and Aikins (5), Gebhardt and Sommer (9), Greenbank (12), Gould (10), Tracy *et al.* (31), Jackson (14), and Thornton *et al.* (29, 30). On the other hand, we have almost always obtained initial potential values for market milks of between + 0.3 and + 0.4 v. Similar levels have been reported by Wilson *et al.* (34), Hobbs (13), Gould (11), Swanson and Sommer (25), Garrett (8), and Webb and Hileman (33). Wide variations in initial potential for samples from individual quarters (+ 0.23 to + 0.38 v.) have been reported by Webb and Hileman, while Swanson and Sommer observed a relationship between the reduced ascorbic acid content and the potential of milks from individual cows. Since the higher levels of potential for market milks have been reported by workers employing vacuum tube potentiometric circuits, while the older type of apparatus was used by all of the group reporting lower levels, we have measured the potentials of a series of six samples from individual cows and one vat sample concurrently, using both Leeds and Northrup Type *K* and Beckman Model *G* (vacuum tube) potentiometers. Although time–potential curves from the former apparatus showed slight fluctuations, apparently due to polarization, the Eh values for both sets of readings were never more than 10 mv. apart. It seems unlikely therefore that these differences in level of initial potential are attributable to the methods of measurement employed. A study of our own data, as well as that of Webb and Hileman, shows no appreciable seasonal differences. We are therefore unable to suggest any explanation for the marked differences referred to above.

Conclusions

Compared to methylene blue, resazurin is much more sensitive to milk from diseased or otherwise abnormal udders. It is equally as sensitive to the reducing activity of bacteria growing in the milk. This greater sensitivity to abnormal milk is regarded as a distinct advantage since such milks can scarcely be regarded as first-class products.

Our findings fail to support the view of Thornton *et al.* that resazurin exerts such a strong poising effect as to complicate the use of this dye or the interpretation of results. There is also no evidence that differences in poising properties of different milks are of sufficient magnitude to affect the results of the test.

Acknowledgments

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References

1. Allyn, W. P. and Baldwin, I. L. J. Bact. 23(5) : 369-398. 1932.
2. Barrett, W. D., Rutan, H., and Keenan, J. A. J. Dairy Sci. 20(11) : 705-709. 1937.
3. Collins, M. A., White, L. M., Chilson, W. H., Turner, H. G., and Rice, J. R. Assoc. Bull. (Intern. Assoc. Milk Dealers) 31 : 104-119. 1939.
4. Davis, J. G. Dairy Ind. 5 : 18-21. 1940.
5. Fay, A. C. and Aikins, G. A. J. Agr. Research, 44(1) : 71-83. 1932.

6. FAY, A. C., MAHONEY, J. L., and WOELFFER, E. W. Rept. Proc. 3rd. Intern. Congr. Microbiol. 1939, 724-725. 1940.
7. FRAYER, J. M. Vermont Agr. Expt. Sta. Bull. 435. 1938.
8. GARRETT, O. F. J. Dairy Sci. 24(2) : 103-109. 1941.
9. GEBHARDT, H. T. and SOMMER, H. H. J. Dairy Sci. 14(5) : 416-446. 1931.
10. GOULD, I. A. Michigan Agr. Expt. Sta. Tech. Bull. 164. 1939.
11. GOULD, I. A. J. Dairy Sci. 23(1) : 37-46. 1940.
12. GREENBANK, G. R. J. Dairy Sci. 23(8) : 725-744. 1940.
13. HOBBS, B. C. J. Dairy Research, 10(1) : 35-58. 1939.
14. JACKSON, C. J. J. Dairy Research, 7(1) : 31-40. 1936.
15. JOHNS, C. K. Am. J. Pub. Health, 29(3) : 239-247. 1939.
16. JOHNS, C. K. Assoc. Bull. (Intern. Assoc. Milk Dealers) 34(11) : 256-265. 1942.
17. JOHNS, C. K. and HOWSON, R. K. J. Dairy Sci. 23(4) : 295-302. 1940.
18. JOHNS, C. K. and HOWSON, R. K. J. Milk Tech. 3(6) : 320-325. 1940.
19. LITTLE, L. J. Milk Tech. 3(5) : 274-279. 1940.
20. MOIR, G. M. New Zealand Dairyman, 43(3) : 55-60. 1938.
21. MOLDAVAN, A. Can. Dairy Ice Cream J. 15(7) : 21-22. 1936.
22. MOLDAVAN, A. Milk Plant Monthly, 27(10) : 58-59. 1938.
23. RAMSDELL, G. A., JOHNSON, W. T., JR., and EVANS, F. R. J. Dairy Sci. 18(11) : 705-717. 1935.
24. RUSSELL, R. R., PALMER-JONES, T., and MOIR, G. M. New Zealand J. Sci. Tech. 21(3) : 161A-167A. 1939.
25. SWANSON, A. M. and SOMMER, H. H. J. Dairy Sci. 23(7) : 597-614. 1940.
26. THOMAS, S. B. and DAVIES, M. Welsh J. Agr. 16 : 231-247. 1940.
27. THOMAS, S. B. and PROBERT, D. Dairy Ind. 6 : 269-270. 1941.
28. THORNTON, H. R. and HASTINGS, E. G. J. Bact. 18(5) : 293-318, 319-332. 1929.
29. THORNTON, H. R., MCCLURE, F., and SANDIN, R. B. Can. J. Research, B, 19(2) : 39-48. 1941.
30. THORNTON, H. R., SANDIN, R. B., and MILLER, C. S. Can. J. Research, B, 13(5) : 257-263. 1935.
31. TRACY, P. H., RAMSEY, R. J., and RUEHE, H. A. Illinois Agr. Expt. Sta. Bull. 389. 1933.
32. WARD, W. E. J. Bact. 36(4) : 337-355. 1938.
33. WEBB, R. E. and HILEMAN, J. L. J. Dairy Sci. 20(1) : 47-57. 1937.
34. WILSON, G. S., TWIGG, R. S., WRIGHT, R. C., HENDRY, C. B., COWELL, M. P., and MAIER, I. Med. Research Council (Brit.) Special Rept. Series, No. 206. 1935.

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STUDIES OF WATERFOWL IN BRITISH COLUMBIA

BUFFLE-HEAD[1]

BY J. A. MUNRO[2]

Abstract

Charitonetta albeola is a common summer visitant to parts of the interior of British Columbia and is abundant on the coast region in winter. Adults arrive in the interior in advance of the yearlings and the sex ratio in early spring flocks is predominantly male. Later, both age groups associate for a short time, then the paired adults become established on nesting territories. Most of the yearling males, and subsequently the adult males, disappear for six weeks or longer. They eclipse and begin to appear again on certain lakes in August but are not plentiful until late September or early October. The yearling females remain on the breeding grounds in flocks throughout the summer. Courtship which is observed first on the coast in March reaches its greatest intensity on the interior lakes in April. Males vigorously defend their nesting territories. Eggs are laid in May and by the last week of June most of the young have appeared. Breeding females leave their broods in order to moult before the young have reached the flying stage and associate with flocks of yearling females that have gathered on certain waters where food is abundant. At this time all are excessively wary. In the interior aquatic insects are the chief food of downy young, adolescents, and adults. On certain lakes amphipods and molluscs are important foods. Fishes, where available, form a minor part of the diet. Seeds of aquatic plants are the main vegetable food except on Okanagan Lake where a larger amount of other plant material is consumed. On salt water, crustaceans and molluscs are first and small fishes, second in importance. The buffle-head is highly regarded as an object of beauty. In the interior it is of economic value as food but is not held in esteem on coast waters. No evidence of it eating commercially valuable fishes was obtained and its consumption of salmon eggs, noted on coast streams, does not reach significant proportions.

Introduction

The buffle-head, *Charitonetta albeola* (Linn.), may be described as a sea duck which undertakes a yearly migration into the interior to nest. In winter it is comparatively abundant on all sheltered coast waters where its tameness, and the handsome appearance of the adult males, make it a general favourite with the observing public; so also in spring their intense sexual activity renders them more than ever conspicuous.

The purpose of this paper is to record the distribution, numerical status, and seasonal movements of the species in British Columbia, as well as the plumage sequence, behaviour, and the food as determined through the study of stomach contents. General observations have been made in this province since 1911 but the greater part of the material presented was obtained in the Cariboo region during the years 1936 to 1941 inclusive.

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Contribution from the National Parks Bureau, Department of Mines and Resources, British Columbia, Canada.

[2] *Chief Federal Migratory Bird Officer for British Columbia.*

It is of interest to note the various similarities between the buffle-head and Barrow's golden-eye, *Glaucionetta islandica* (Gmelin) (6). Their summer distribution in British Columbia, both geographically and in respect to habitat, is identical; so also in place and time are their migratory movements. Courtship behaviour follows the same general pattern; both are tree-nesting ducks and the downy young of the two species are closely similar. The same sequence of moults is followed, their seasonal movements, the local distribution of different age groups and general behaviour on the nesting grounds are much the same.

Distribution, Seasonal Movements, Summer Populations

Summer

The buffle-head nests in the interior of the province east of the Cascade Mountains, the centre of abundance being the dry belt region comprising the Nicola, Kamloops, Cariboo, and Chilcotin districts. It is less abundant in the Okanagan, reported as common on the Arrow Lakes (4) and scarce in the East Kootenay (W. B. Johnstone, personal letter). It nests at Ootsa Lake (J. L. Shelford, personal letter) and is recorded as breeding at Hazelton (7) and in the Peace River district (2). It is not recorded from the Skeena region (10) nor from the Stikine region (9) but is a regular and fairly abundant migrant at Atlin (11).

Spring Migration

A migration from the south and from the coast takes place in March and April. Known migration routes are the Columbia and Okanagan Valleys, the Fraser and Thompson river system, the Harrison, Lilloet, and Seton lake system and the Cheakamus river and lake system. Undoubtedly many other rivers farther north are fly ways into the interior.

In the Okanagan and Nicola regions the spring migration reaches its peak in the latter part of April or early May when on favourable lakes of small size the aggregate of birds (that seldom are together in one flock) number 25 to 50. The following counts represent maximum numbers seen.

Falcon Lake	—	April 14, 1940: 40.
Munson's Lake	—	April 19, 1934: 53; May 8, 1937: 47.
Trapp Lake	—	April 19, 1933: 33.
Rawlings Lake	—	April 24, 1937: 25; April 12, 1940: 20.
Tule Lake	—	April 25, 1934: 40.
Swan Lake	—	May 11, 1933: 57.

Most of the birds in these flocks are transients and all except a relatively small number move out before the end of May.

Farther north in a more thickly populated nesting region concentrations have been observed on 103 Mile Lake where exceptionally favourable food

conditions exist, but the average spring flocks are of similar size to those seen elsewhere.

103 Mile Lake	—	April 16, 1941:	250.
105 Mile Lake	—	April 16, 1941:	50.
Cummings Lake	—	April 18, 1941:	56.
Mirage Lake	—	May 11, 1939:	37.
Beaver Dam Lake	—	May 10, 1939:	40.

There is still at this time a considerable number in small flocks moving up the coast; in April this movement includes both adults and yearlings usually in separate flocks. Adults migrate inland in advance of the yearlings and by the middle of May the latter predominate in the coast flocks. This was the case at Massett, Queen Charlotte Islands, May 1 to 20, 1920, where daily observation of the population revealed a steady reduction in the number of adults as the month advanced, so also at Tlell, Queen Charlotte Islands, where on May 4, 1935, the population consisted of three adult males and 22 females, which were considered to be yearlings. The latest recorded date of spring migrants at Port Hardy, Vancouver Island, is May 8, 1940 (Allan Lyon, personal letter), at Tlell, May 14, 1935, and at Massett, May 20, 1920.

Segregation of the two age groups has been observed early in the spring, as at Hammond Bay, Vancouver Island, March 28, 1941, where a flock of 37 adult males and 32 females, also probably adults, contained no yearling males. That the two age groups separate while on the coast and migrate inland at different times is also suggested by observation of spring flocks in the interior. Thus of more than 500 examined in the Kamloops and Cariboo regions April 14 to 18, 1941, all the males and, so far as could be determined, all the females were adults. One yearling male was seen April 23 and two on April 24; a month later yearling females were plentiful and yearling males were seen daily.

Summer Populations

During late April and early May an association of adults and yearlings on the nesting grounds is general. The membership of these associations is constantly changing; adults pair off and move elsewhere, so also the yearling males, now rapidly becoming lighter in colour and more conspicuous, gradually disappear. Later the numbers of a flock may be temporarily increased by the arrival of males that have fulfilled their part in the reproductive process. At the same time other males, probably mated later, are still conspicuous in appearance and behaviour on their nesting territories. Thus at 103 Mile Lake, June 3, 1937, 15 adult males dived for food, rested on the water or dressed their plumage in company with 30 yearling females, while the same day on adjacent 105 Mile Lake mated males were actively displaying, rushing at each other across the water, and making nuptial flights around the lake. Other dates for the early flocking of postbreeding males on the nesting ground are: Westwick Lake, June 3, 1937, 30 adult males in a total population of 50; Hunter's Lake, south of Kamloops, June 11, 1938, 17 males in company with

five faded yearling females. The latest dates for the appearance of postbreeding males prior to the summer moult are: July 3, 1938, when 11 on 103 Mile Lake and six on 105 Mile Lake were counted, and July 11, 1938, when a single male was seen.

Following the brief stay of the postbreeding males with the yearling females, there may be a short period when the rafted population is composed exclusively of the latter age group. Some associate in small bands up to 15 or 20; others, perhaps the greater number, assemble on certain lakes, not always those which spring flocks have occupied, and remain there until after the moult in August. These serve as nuclei to which are attracted first the local breeding females and then adolescent young, then postbreeding females that have abandoned their broods on other lakes of the region and finally, towards the end of the summer, moulting adult and yearling males that have renewed their flight feathers.

The most notable concentrations of non-breeding females, with later additions of adults and young, were observed at 103 Mile Lake. The physical characters of this lake and the behaviour of the rafted ducks as observed in 1936 have been described elsewhere (5). Subsequent observations are briefly summarized as follows.

On June 2, 1937, 40 individuals of which 15 were adult males and the remainder yearling females were together in one flock. On August 4, the population had increased to 500 (estimated), of which most were considered to be non-breeding yearling females. Some were flightless but the majority took wing at 150 yards or at an even greater distance when approached by a canoe. A number of flightless birds examined at close range through binoculars were faded to light drab on head and flanks. This association had almost completely dispersed by August 25 when only 20 were on the lake; these were chiefly young that had not attained full power of flight.

The population on July 3, 1938, consisted of 10 adult males and 11 yearling females and on July 26, of approximately 130 females and one yearling male. The latter was losing its flight feathers and able to fly only a short distance. On August 16 the number was reduced to 40, most or all being yearling females.

No concentration took place in 1939, and on July 18, the population consisted of two adult females with a total of eight young.

On June 12, 1940, a total of 12, of which eight were adult males, was present; these were associated with Barrow's golden-eye. On August 6, approximately 250, all females, comprised the largest proportion of a raft of diving ducks which otherwise was composed chiefly of Barrow's golden-eye and lesser scaup ducks, *Nyroca affinis* (Eyton). When disturbed, as they were half a dozen times, the buffle-heads rose in small bands and after circling once or twice, left the lake.

Other summer concentrations have been observed, for example: 64 at Mirage Lake, August 4, 1938; 75 at Tunkwa Lake, August 9, 1939; 300 at Minnie Lake, August 10, 1939.

At Rush Lake, Springhouse, Cariboo, between June 13 and July 10, 1941, the number of postbreeding males and second year males and females changed from day to day. The adult males were in full breeding dress, the second year males in various stages of moult with varying amounts of white on face and back. Some rose heavily from the water but all were strong on the wing and exceedingly difficult to approach. For several days one of the yearling males accompanied a female with brood of downy young. The changes in this population are shown in the following counts.

	Adult ♂	Yearling ♂	Yearling ♀
June 13	3	5	4
June 14	0	3	2
June 15	5	7	0
June 18	4	6	2
July 7	0	1	6
July 10	0	0	12

A female with brood of five, about one-quarter grown, were present on June 13. Other females with broods of downy young as indicated appeared on the following dates, 11 on June 20, 6 on June 23, 4 on June 24, 8 on July 7.

The following are examples of typical summer populations on small breeding lakes.

On Tatton Lake, June 11, 1940, the population consisted of three mated pairs, three females with broods of downy young numbering 5, 6, 7, and 12 yearlings of which 10 were females. On August 3, 1940, the total present was 50 (estimated) consisting of adult and yearling females and young of the year.

On 105 Mile Lake, June 12, 1940, the population was three mated pairs and one female with brood of eight downy young. On August 5, 1940, a total of 40, most of which were associated with a raft of lesser scaup duck, included full grown young, adult and yearling females, and three adult or yearling males in partial eclipse.

On the nesting grounds adult and yearling males are scarce or absent for approximately six weeks from early July to mid-August. In 1936, none was seen and in 1937 five were recorded as follows: two yearlings, Disputed Lake, July 25, in company with two yearling females; two probably adult males, Green Lake, July 27, one completely flightless, the other capable of fluttering over but not rising from the water; one flightless bird in partial eclipse, 103 Mile Lake, August 4. In 1938, one partially moulted yearling male, 103 Mile Lake, July 26, was the only record; subsequently none was seen until late August. No males were recorded in the summer of 1939 until August 22 when the first of those that had renewed their flight feathers appeared on 103 Mile Lake.

In the summer of 1940 adult and yearling males were more conspicuous on the nesting grounds than had before been observed. The following are definite records:

105 Mile Lake, Aug. 5: 3;	148 Mile Slough, Aug. 9: 1;
Cummings Lake, Aug. 10: 1;	Watson Lake, Aug. 12: 1;
103 Mile Lake, Aug. 14: 2;	Tatton Lake, Aug. 14: 1.

That this general absence of males from the nesting grounds covers roughly the period of moult and that the period is short, a month to six weeks, suggests that they retreat to waters not far distant. It is suggested that eventually important summer concentrations of males will be found on some of the ornithologically unexplored lakes in central British Columbia. Very likely they will be in large flocks as are most of the yearling females at this time and, judging from the actions of those few summer males that have been encountered, they will be exceedingly wary.

Autumn Migration

Small numbers of moulted adult and yearling males reappear on the nesting grounds in late August but in some years they are uncommon, even as late as the last week in September. Thus at Longbow Lake, September 23, 1940, only three were detected amongst three flocks totalling 150 and at Irish Lake, September 25, 1940, a flock of 50 contained only one. A total of 22, 103 Mile Lake, August 20, 1937, and five, 105 Mile Lake, August 22, 1939, represented exceptionally early appearances.

Adult males usually do not appear in numbers until October and at no time do flocks on the interior lakes contain the same percentage of adult males as do winter flocks on the coast. It seems likely that an early migration of males from the interior to the sea occurs in advance of the main migration, as in the case of the lesser scaup duck.

A limited southern movement from the nesting grounds in the Cariboo occurs in September but not until the first freeze-up, which usually takes place in late October, is there a general exodus. Following this the numbers on the lakes in the southern interior are increased by the arrival of these migrants. Later, as winter approaches in these lower latitudes, the bulk of this transient population moves south or west to the coast. A series of counts on Swan Lake, Okanagan, illustrates the rise and decline in numbers at this time:

1933 — Nov. 1: 100; Nov. 4: 200; Nov. 17: 50;
1939 — Sept. 30: 250; Oct. 28: 400; Nov. 18: 200.

Winter Population

Fewer buffle-head now winter on Okanagan Lake than 20 odd years ago. In the winter of 1915–16, 30 to 40 frequented the lake near Okanagan Landing even after a prolonged period of subzero weather (January 10 to 19) froze a large part of these waters; approximately the same number remained the following year. In the winter of 1917–18, five was the largest number seen in one day (January 26, 1918) and since that time it has been scarce or absent

on this lake during winter. No definite information is available concerning other possible winter populations in the interior.

The first autumn arrivals on the coast appear usually in September; a record of two females, Della Lake, Vancouver Island, August 20, 1923 (8) is considered unusual; the earliest recorded date at Port Hardy is October 5, 1939 (Allan Lyon). The numbers of this partially transient autumn population on the coast reaches its maximum in November. At this time small flocks are plentiful at many places and concentrations take place on particularly favourable feeding grounds; 200 at Esquimalt Lagoon, November 11, 1938, 80 at Shoal Harbour, November 12, 1938, 450 at Sooke Harbour, November 14, 1938, are examples.

Later in the autumn there appears to be a southerly movement so that the winter population in south coastal British Columbia is not as large nor as concentrated as it is during November. During the period December–February an average flock on southern Vancouver Island contains a dozen or so birds; the number of flocks, widely distributed on both salt water and fresh, and the total of individuals is greater than at first appears. For example the total of a number of flocks at Telegraph Bay, December 12, 1940, was approximately 150. It was the duck most frequently seen along the six miles of coast between Sooke Harbour and Tugwell Creek on December 11, 1940, and thereafter until March 17, 1941.

The population frequenting Departure Bay in winter varies from eight to 15 and when the herring begin to spawn in early March suddenly increases to 100 or more. Concentrations are apparent elsewhere at this time; 75 at Esquimalt Lagoon, February 25, 1934, 84 on March 28, 1938, 99 on March 17, 1939, 150 at Camp Slough, Chilliwack, March 29, 1935, are examples.

TABULATION OF SEASONAL MOVEMENTS

January–February	Association of all age groups on coast waters
March–April	Migration of adult population to interior
April–May	Migration of yearlings to interior, small flocks assemble on interior lakes; adults mate
June	Adult and yearling males disappear; young are brought to water
July	Populations restricted to adult females with young, yearling females and an occasional adult or yearling male
August–September	Concentrations of moulting adult and yearling females joined by flying young and by a few adult males and second year males now in adult plumage
September–October	All age groups assemble on suitable feeding grounds; commencement of migration
October–November	Migration south and to coast
November–December	Association of total population on coast waters

Reproduction

It seems probable that buffle-heads do not breed until their third year. The chief evidence for this is that large numbers of second year females associate in flocks on the breeding grounds at the time when breeding females are incubating or caring for young. Some inconclusive evidence suggesting exceptions to this rule follow.

A nesting female under daily observation on Boitano Lake, June, 1941, was conspicuously lighter in colour than normal breeding females, a condition indicative of yearling females at this season as explained elsewhere in this paper.

On a small mountain lake near Summerland, Okanagan, May 11, 1918, a nesting female was apparently mated to a male in second year plumage modified by extensive white areas on the face and the amount of white on flanks and shoulders characteristic of many yearlings in late April and May. The female joined this male each time she came to the water and in general the two acted as if mated; there were no other buffle-heads on the lake. Instances of yearling males associating in the nesting season with a particular female, in some cases where no other buffle-heads were present, also have been noted. For example on a pond near Vernon, June 8, 1928, and on Westwick Lake, Cariboo, June 17, 1941.

Courtship

Courtship display is first noticed on the coast in late January or early February and reaches its greatest intensity in the interior during April. At this time on the lakes where buffle-heads concentrate during the courtship period, the number of adult males usually exceeds the number of adult females; few second year birds are then present. At 103 Mile Lake, April 16, 1941, where the greater part of the local population was assembled, at least 175 of the total of 250 were adult males; some had paired, there were many trios (2♂, 1♀) and units of eight or 10 males accompanying two or three females. In one case eight males flew after a female and when the flock alighted four of these males rushed over the water towards the female. The males are exceedingly hostile toward one another. A mated male will swim under water toward an intruding male, emerging momentarily for direction if the distance separating them is considerable and then attempt to come up beneath the male on the surface so that it is obliged to rise. Should the second male alight again in the vicinity, the underwater attack is repeated. In one instance observed this performance was carried out by one male a dozen or more times, directed in each case against the same intruder. When the latter finally retreated the mated male swam toward the female, uttering a soft cooing note.

Courtship behaviour varies in detail and intensity; that of seven adult males, one yearling male, and two females was restricted to bobbing the head and a continuous "cooing" by several of the adult males. After five minutes

the flock dispersed and commenced feeding, four mated birds remaining together.

The behaviour on a small pond of four males that courted one female for 30 min. without interruption (April 22, 1941) was full of action and excited movement. Each of the males was in constant motion about the female; one that appeared to be her mate stopped displaying every few minutes and rushed over the water at one of the others. Four different actions were performed by the males.

1. A quick glide past or around the female, head bobbing rapidly.
2. A flight of a few yards, in which the pink feet were displayed; then a slide across the water, body partly upright and chest outthrust.
3. The male standing partly upright splashed the water with his wings, or, in a horizontal position, splashed alternately with wings and tail.
4. Bowing with vigorous forward thrusts that immersed the head.

All executed one or another of these actions simultaneously, the tempo rising to reach an excited turmoil which lasted for a minute or so and then subsiding to less sustained effort. The female swam quietly about the pond and to these demonstrations made no response. At another place the female of a pair danced in a partly upright position about the male; the latter did not respond.

Courtship actions on the water are interrupted by display flights conducted by a group of males, by single males, or by both sexes together. Thus at Rawlings Lake (April 12, 1940) five adult males and one female rose from near the centre of the lake, which is half a mile by one-quarter mile, and after circling most of its circumference in rapid flight, splashed into the water about 40 yd. from shore. In descending, the rose-coloured feet of the males were conspicuously displayed. Immediately after alighting all commenced to move across the water in a manner of progression that can best be described as a series of hops. In these movements the body was partly raised from the water and the wings half spread so that a continuous splashing took place. A single chuckling note was repeated in rapid succession, apparently by the males only. After several minutes in which this performance was continuous two other males joined the group and at once commenced to act in the same manner. Sometimes the single female in the group travelled ahead of one or more males and sometimes she followed them. Sometimes a male seized another male by the feathers on the back neck and held on while the lower male submerged as in copulation. These energetic and excited movements in which all took part continued for about five minutes, then all rose and alighted again about a hundred yards from shore where they began diving for food. Even while so engaged the urge to display was insistent and the males, upon rising to the surface, invariably started jerking their heads up and down in the courtship bow.

In the full tide of sexual excitement the awareness of the male for the female is dominant, greater than their reaction to danger. Thus at Swan Lake

(May 2, 1936) the author was paddling toward a trio of two males and one female which were swimming close together along the edge of the rushes. When the canoe was 70 yd. distant, the two males rose but the female continued swimming. A few moments later one of the males, after flying a quarter mile up the lake, turned around and headed straight toward the female. When 20 yd. or so from the canoe, with rosy feet advanced and wings down-curved, he checked his speed and dropped to the water beside her. A moment later the female rose and the male quickly followed.

Second year males may exhibit some of the pugnacity so characteristic of the adult male. Thus at Okanagan Landing (May 4, 1919) a young male which accompanied two mated pairs repeatedly attacked one or another of the older males.

Courtship display continues during the laying period and terminates abruptly when the males leave their territories to associate with other males and non-breeding females. At this time the cessation of intense, almost constant sexual activity and the tolerance of one male for another is in marked contrast to earlier behaviour.

Nesting

The dry, semiopen Cariboo region supports the largest summer population. Here pairs are found in occupation of alkaline ponds, sloughs, and small lakes; they are less attracted to large lakes and to those situated at altitudes above 4000 ft. The high mountain ponds sometimes occupied by breeding Barrow's golden-eye are seldom used by the buffle-head.

A breeding pair establishes a definite territory which the male vigorously defends from encroachment by other males. The territory may be an entire pond or, on larger waters, an area along several hundred yards of shore line. In the latter part of May and in early June, males may be seen alone on these territories during the time each day when the female is on the nest. In some cases at least the male is aware of the location of the nesting tree and the whereabouts of the female during her absence from the water, as is suggested by an incident that took place on a small lake, May 15, 1916. A nesting tree was hit noisily with a stick several times and before the female appeared at the entrance a male rose from another part of the lake and splashed into the water beside the tree.

An incubating female does not readily leave the nest even after the nesting tree has been rapped several times. One has been seen to thrust her head and neck through the entrance and remain in that position for several minutes before flying out. It was observed on another occasion that a female after being flushed from her nest returned to it immediately when the observer had left its vicinity. While egg-laying continues the male constantly companions the female during the time she is on the water; he is still violently excited by the appearance of other males and the sexual manifestations described above continue.

By the time the females have brought the young to the water it is not usual to find males on the nesting lakes unless there has been considerable difference in time between the laying of individual birds. This perhaps was the case at Tatton Lake, June 11, 1940, when a male joined a female with brood of downy young and remained with her for some time. This was interpreted to be the sexual reaction of a male whose mate was in the egg-laying stage of reproduction.

So far as known nests are invariably in trees, more often in dead trees standing in or close to the water. For this reason nests of the buffle-head are easier to find than those of Barrow's golden-eye which may be a mile or more distant from the lake where the female will later lead her young. The buffle-head nests that have been studied were located either by keeping a breeding female under observation until she flew to her nesting tree or by detecting the presence of down at the entrance to the nest. Old nesting holes of flickers that have enlarged with the tree's decay supply the necessary requirements. Two sites found beside a small alkaline lake in the Okanagan are characteristic.

The first was in a dead Douglas fir 8 in. in diameter that had been broken off 30 ft. from the ground. It stood on the lake margin and leaned toward the water; the top was hollowed to a shell of bark. The entrance to the nest was four feet from the top of the tree and one foot above the bottom of the cavity. On May 22, 1915, this contained 10 partly incubated eggs insulated by a large amount of down. Beneath the down, mixed with the rubbish at the bottom of the cavity, were shell fragments of eggs laid in a previous year.

The second, which contained nine fresh eggs on May 15, 1916, was near the top of a 30 ft. yellow pine stub standing in a foot of water six feet from shore.

At Cummings Lake, June 9, 1941, a Douglas fir stub 10 ft. in height standing in three feet of water 20 ft. from shore contained a nest and six eggs. The entrance, of irregular shape and 4 in. wide at the widest point, was five feet above the water; the nest cavity measured 6 in. by 8 in. (Fig. 1). When a canoe touched the bottom of the stub the female flew out and alighted on the water 50 yd. away.

At Boitano Lake, June 10, 1941, the actions of a female indicated that a nest was in the vicinity. She swam in a nervous manner back and forth close to shore across a space of 30 yd. or so and at short intervals uttered a barely audible nasal quack. The location of the nest, which contained nine eggs, was soon determined by the finding of fresh down on the rough surface of a poplar stub a short distance from the lake (Fig. 2). The stub was 14 in. in diameter at the base and had been broken off 10 ft. above the ground; the lower portion had rotted so that it moved at a touch and ants had used it as a backing for an accumulation of fir needles and rubbish. The entrance to the nest measured 3 in. in diameter; the nest cavity, a flicker's excavation several years old, measured 8 in. across and extended 12 in. below the entrance; the wood at the entrance and the interior walls were solid. In order that a

person's hand and arm could be inserted it was necessary to enlarge the entrance by whittling the hard wood with a knife. The placing of a ladder against the tree, the enlarging of the entrance to the nest, and the taking of measurements and photographs occupied over half an hour; during all this time the duck remained on the nest.

The stub was visited on each of 11 successive days. Except once when the buffle-head left while the observers were approaching, and another time when she was absent, the procedure was to strike the stub several sharp blows in an endeavour to make her leave the nest. This did not succeed in its object but on three occasions she left precisely 10 min. after exploring fingers had touched her on the back and head. Each time she covered the eggs before leaving. On other days her presence was determined by touch and she did not leave while the observers were in the vicinity. With one exception these visits were made between 8.00 a.m. and 9.15 a.m.; the exception was at 4.00 p.m., this being the occasion she was not on the nest. On the four mornings she was seen leaving the nest she flew directly to the lake and remained there an hour or longer. As circumstances did not permit continual observation, it was not possible to determine whether she had regular hours for feeding.

The stub stood 100 ft. from the water near the edge of a fir and poplar wood. Nearby a rail fence extended into the water; the lower rail, 150 ft. in a direct line from the nest, was used as a resting place between periods of feeding. The feeding ground, limited to an area roughly 50 yd. by 100 yd. and no doubt approximating the area of the nesting territory, was directly in front of this point (Fig. 3).

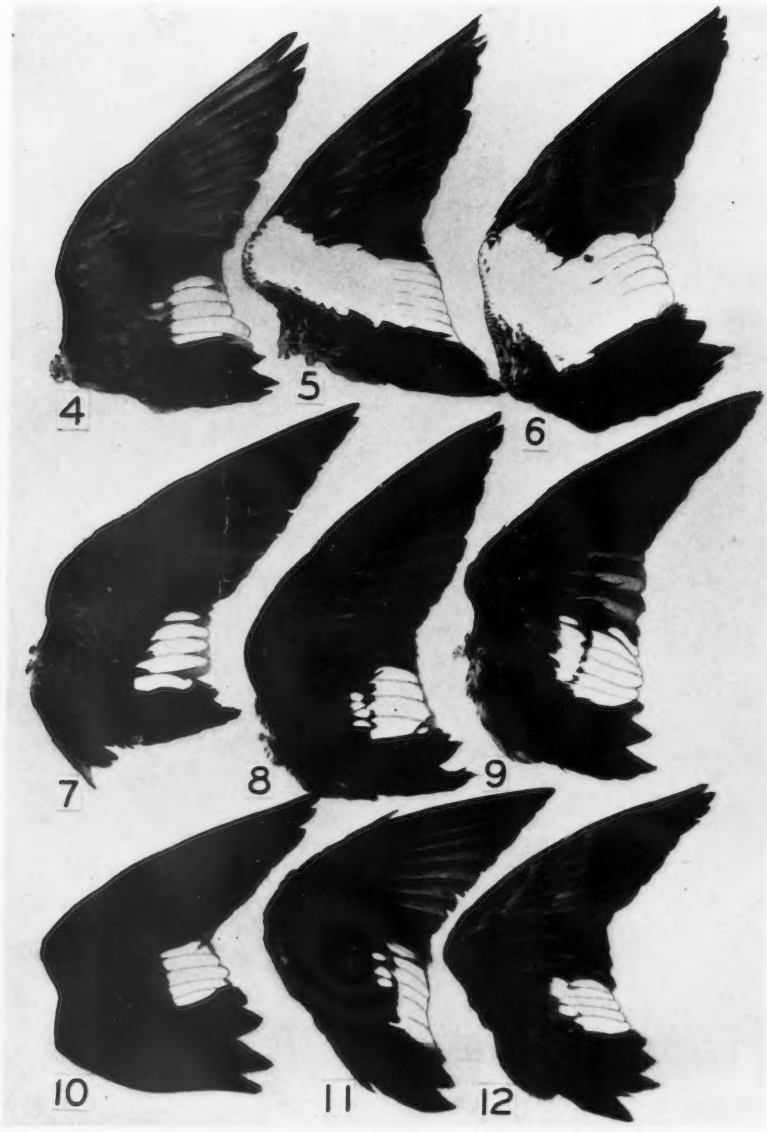
When the stub was last visited at 2.30 p.m., June 23, all but two of the eggs had hatched; one contained a dead embryo, the other was cracked and the contents dry. The young were gone. The female, recognized by the faded pattern on her upper parts, and six young were seen the same day on Rush Lake which is about 200 yd. from the nearest point of Boitano Lake. The former is well stocked with food, the latter is a "soda" lake containing no vegetation and an animal population restricted chiefly to phyllopods and corixids.

In view of the few references in literature to the actions of nesting buffle-heads the following minutiae of behaviour as observed from a concealed position on the morning of June 12, 1941, are submitted.

8.20. Buffle-head flew from nest and alighted on water 60 yd. from shore where she commenced feeding. During the next hour she was visited by another female buffle-head, probably a yearling, and later by an adult male and four yearling females. At 9.20 when an observer visited the nesting stub, the duck moved in close to shore and swam back and forth quacking softly. After 9.45 she was kept under close observation through 6× binoculars until she returned to the nest at 11.19.

9.45 to 10.05. Remained on water directly in front of nest within an area of approximately 200 sq. yd. of which the inner boundary was about 30 yd.

FIG. 1. *Douglas fir stub containing buffle-head nest, Cummings Lake, British Columbia.* FIG. 2. *Poplar stub containing buffle-head nest, Boitano Lake, British Columbia.* FIG. 3. *Nesting territory of buffle-head, Boitano Lake, British Columbia, nesting place in poplars at left, resting place on fence rail at right, feeding ground on lake at centre.*



FIGS. 4 TO 12. *Wing pattern of buffle-head, approximately ½ life size.* FIG. 4. ♂, *age approximately seven months.* FIG. 5. ♂, *age approximately 13 months.* FIG. 6. ♂, *adult.* FIGS. 6 TO 8. ♂, *immature, variation in pattern of greater wing coverts.* FIGS. 9 TO 12. ♀, *variation in pattern of greater wing coverts.*

from shore. Here she dived for food or floated on the surface sometimes dressing her plumage.

10.05. Swam to shore and climbed on to the lower rail of fence that slanted into water 10 ft. from the beach. She stood upright and kept shaking her tail vigorously, stretched out one wing then the other, ran her bill through the lower body plumage, the flank feathers, and under the partly opened wing. When not engaged in these movements she maintained an alert position, neck extended and head moving from side to side. At no time did she relax.

10.15. Turned sideways on rail and assumed horizontal position, then flew 75 yd. out on lake where she commenced feeding. She dived three times remaining under 14, 18, 18 sec. with intervals on the surface of 15 and 8 sec. Remained on surface 90 sec. preening plumage and shaking tail while in a horizontal position then standing partly upright and shaking wings. Two dives of 12, 13 sec. with interval of 15 sec. on surface; for 97 sec. she drifted motionless.

10.20. Flew to fence rail, climbed up and resumed dressing of plumage with constant vigorous tail shaking; alert as before.

10.27. Flew to feeding ground, submerged once then rose, made a circle over lake and alighted close to shore in front of nest site; remained there five seconds then flew to top of fence post on beach where she remained 35 sec. moving head from side to side.

10.30. Alighted on water again 20 yd. from shore; remained there 50 sec. then flew out on lake 75 yd. and commenced feeding. The length of time below the surface in four dives was 12, 15, 15, 17 sec., the length of time on the surface, 6, 6, 8 sec. After the final dive she stood up, shook her wings, and dressed her plumage. On the surface for 30 sec. She then rose, circled over the beach towards the nest, and returned to the water.

10.35. Flew towards nest site, returned to lake, and alighted near fence.

10.37. Climbed on fence rail and for 8 min. continued the activities described.

10.45. A female Barrow's golden-eye swam to fence rail; the buffle-head dropped to the water and swam out 50 yd., dressed plumage for a few seconds then flew straight towards nest site but turned and alighted on water.

10.47. Swam towards fence rail now occupied by female Barrow's golden-eye; swam in a circle about the other duck, drank several times; stood up and shook her wings, then flew to a position on the fence rail three feet from the golden-eye. Continued dressing plumage, stretching and shaking tail (all of which activities were also being practised by the golden-eye).

10.55. Flew to position 50 yd. from shore, rose immediately and flew towards nest, returned and alighted near shore.

10.57. Flew 40 yd. farther out and continued to feed for 3 min., then floated on the surface sometimes standing up to shake the wings.

11.04. Started to swim inshore towards fence rail.

11.07. Climbed to place on rail vacated by golden-eye; more tail-shaking and wing-stretching.

11.08. Slid off rail, flapped wings, and in 30 sec. climbed up again. For 11 min. she continued the activities described above but less vigorously and with longer intervals of alert watchfulness.

11.19. Flew directly from rail to nest.

Behaviour of Females and Young

The majority of the buffle-head broods appear on the water in June, the earliest record being June 12, 1940. The average number in a total of 58 broods, counted in July 1938 and July 1939, was seven. Females react to alarm in various ways; some are attentive parents and remain with their broods even after becoming excited by a threatened danger. For example, on Bridge Creek, July 13, 1937, when a female with eight half-grown young was overtaken on the narrow stream by a passing canoe she did not rise from the water nor increase her swimming speed beyond the ability of the young to keep up. When a family is isolated, as was the one mentioned above, brood formation under the care of a female is likely to be maintained until the young are well grown, but on well populated lakes females show less concern and if alarmed are likely to fly and leave the young to fend for themselves.

Thus on a small lake with a relatively large population broods tend to lose their identity while the young are still quite small. Under such conditions a thorough mixing of the population takes place; one female may have one or two young and another a dozen; one instance of a female with 18 young has been observed. In a population such as this some may leave their broods unattended and swim about in company on another part of the lake. The uncared for young may join other broods, remain together as a brood, or dissolve into several units. This condition has been noticed particularly on lakes where the surface is covered with lily pads or a heavy growth of *Potamogeton* or other similar plants. By reason of such obstruction the small young are not always able to keep up with the female, they are left behind and eventually become lost. Single young thus abandoned may wander for some distance and even leave the water as did a young bird about a week old which was captured (July 20, 1937) beside a road through thick woods one mile from the nearest water. When adolescent young are attracted to a raft of non-breeding females, they cease to maintain the brood formation and become individual members of the larger association.

In some cases the scattered, half-grown young, or even compact broods, are deserted permanently. Thus at Lily Pad Lake, August 7, 1937, only three broods of 5, 7, and 8 were accompanied by females while 2, 4, 5, 5, 6, and 10 young were unattended. The same condition was observed there on July 21, 1938, when four females accompanied broods of 1, 2, 3, and 5, and a total of 19 young, i.e., 1, 1, 1, 2, 3, 3, 4, 4 were not accompanied.

Broods sometimes are accompanied by two females: for example, at Buffalo Lake, August 7, 1937, this was so in the case of a brood numbering seven. As with Barrow's golden-eye (6), the second female usually is a yearling.

There is some fraternizing on the nesting grounds between the buffle-head and other ducks. It is not uncommon to see a female lesser scaup duck, usually a yearling, associating with a female buffle-head and brood while sometimes a yearling female buffle-head is seen with a brood of lesser scaup ducks.

Young buffle-heads sometimes follow a female, or male, of another species. Thus at 103 Mile Lake, August 8, 1936, a brood of 10 nearly full-grown young followed closely behind an adult, flightless male white-winged scoter, *Melanitta deglandi*, and continued to do so until the scoter dived and was not seen again. They also attract, or follow, broods of other species. For example, at Cummings Lake, August 10, 1940, two half-grown buffle-heads accompanied a brood of five lesser scaup ducks and at 105 mile Lake, August 5, 1940, a brood of nine was followed by five half-grown Barrow's golden-eye.

Sex Ratio

Determination of sex ratio is complicated by two factors (1) the similarity during autumn and early winter between males in first year plumage and females, (2) a partial segregation of adult males in early spring. In studying the sex ratio of autumn and winter flocks it has been observed that the number of adult males represented about 25% of the total. This was the case on November 6, 1940, in nearly all of 20 small flocks counted along 30 miles of sea coast between Departure Bay and Qualicum, Vancouver Island. If it is assumed that young males were present in about the same proportion as adult males a balanced sex ratio is indicated.

Counts made at the coast in March, when the identification of second year males presents little difficulty, show a preponderance of males:

		♂	♀
Esquimalt Lagoon	March 28, 1938	48	36
Departure Bay	March 25, 1938	60	40
Departure Bay	March 20, 1940	80	50
Hammond Bay	March 28, 1941	37	32

The following counts to determine the sex ratio were made on lakes where mating birds concentrate and the constitution of the flocks is constantly changing. It is possible that the excess of males indicated is a temporary seasonal condition corrected later with the arrival of additional females. On the nesting grounds during April adult males may outnumber females five or six to one; at this time few second year birds are present.

		♂	♀
103 Mile Lake	April 16, 1941	175	75
105 Mile Lake	April 16, 1941	35	15
Westwick Lake	April 18, 1941	10	4
Cummings Lake	April 18, 1941	40	16
Slough, 108 Mile Lake	April 22, 1941	16	4
Trapp Lake	April 24, 1941	16	4
Napier Lake	April 24, 1941	26	5

Moults and Plumages

In connection with this study 64 specimens in the author's collection, selected to represent both sexes from downy young to adult, have been available for reference. The specimens referred to below, by number, are in this collection. In addition, 122 other specimens comprising the collections of the Provincial Museum of British Columbia, the Royal Ontario Museum of Zoology, and the Canadian National Museum have been examined. The following is an attempt to interpret the plumage sequence on the basis of this material. The colour terms are those of Ridgway[1].

Downy young. The downy young buffle-head is white below and fuscous above with white markings on wings and white cheek patches; the fuscous colour of the natal down soon fades to a lighter, more brownish shade and by the time the first teleoptile shafts appear it has become still paler. In general appearance it closely resembles the similar stage of *Glaucionetta;* a constant differentiating character, perceptible only in freshly taken specimens, being the colour of the foot which in *Charitonetta* is dark plumbeous and in *Glaucionetta* deep olive.

♂ *Juvenals, July–August.* Chest, neck, upper tail coverts, and sides of head nearest to hair brown; indistinct white patch on cheeks and indication of white on throat; crown and interscapular region fuscous, intervening area on neck paler; wings black, except for larger secondaries and white markings on middle secondaries; larger secondaries olive-brown fading to drab and forming distinct *V* on back; tail fuscous, fading to pale brown; flanks and lower tail coverts hair brown to drab; lower chest and belly white. Fresh colours are evanescent and quickly modified by breaking down of the feather structure.

♂, *First autumn and winter, September–February.* During the first autumn the rectrices and contour feathers are gradually renewed. No. 4732, September 28, 1936, is in a state of complete body moult, the new rectrices about half-grown. The head becomes fuscous, the cheek patches are larger and more clearly defined than before; the chest is lighter in colour, pale smoke gray feathers with white edgings; the back is uniformly black, fuscous or deep

[1] *Ridgway, R. Color standards and color nomenclature. Published by the author. Washington, D.C. 1912.*

mouse gray with feathers on interscapular region tipped with lighter shade; flanks are solidly mouse gray; the tail, nearest to neutral gray, has a silvery sheen.

Some individuals retain this plumage with little modification until well into the winter. Thus No. 4518, January 12, 1935, is similar to specimens taken in October except for the presence of several white flank feathers. Others as early as November show modification brought about by moult. For example No. 5194, November 13, 1939, is conspicuously lighter than the January specimen referred to, a condition produced by the presence of white feathers on scapular regions and head, and white and light grey feathers edged with black on the flanks; this specimen also has conspicuous violet and bronze feathers on the head.

♂, *Spring and summer, second year.* As spring advances they become lighter in colour partly through the appearance of white feathers on flanks, scapulars, and head, partly through the breaking down and fading of the old plumage. Later, white middle wing coverts appear and these with the white middle secondaries form a conspicuous bar across the black wings (Fig. 5). The remiges become much abraded and fade to drab; in some they degenerate to a condition that impedes or prevents flight.

Three specimens in the author's collection, No. 5423, June 20, 1941, No. 5424, June 27, 1941, No. 5425, July 7, 1941, are in a full moult of the body plumage. On the ventral surface the vanes have broken down revealing the darker basal portions and shafts of the feathers so that the colour in the mass is pale smoke gray rather than white; in each the throat is white or buff, the chest drab gray or smoke gray, the rectrices, primaries, and secondaries light drab; the tips of the feathers on the interscapular region are faded, in one specimen forming a regular pattern. All these modifications have been produced by feather disintegration which also has altered the character of the violet-green feathers on the head and darkened the white areas of the same region. New feathers appearing on the lower chest and belly are white, those on the upper chest are light mouse gray, and those on the flanks are deep mouse gray. The residue of old feathers on the flanks is pale gray, white, and white edged with black. In the tail of one specimen, in which some of the abraded rectrices have broken off, is one new adult feather.

At the time these specimens were taken the few adult males present showed no indication of moult.

Three other specimens, No. 5009, August 4, 1938, No. 5281, August 8, 1940, and No. 5178, August 23, 1939, have faded on head, flanks, tail, and back to such an extent that the entire dorsal surface is mottled buff and brown of various shades. In one, the wings feathers have been shed and the new primaries are one inch or less out of their sheaths. In none is there a recognizable indication of eclipse plumage. After the second year moult the young male is probably indistinguishable from older males.

♂, *Summer plumage.* The five specimens described above are identified as being in their second year. The age of seven other specimens taken later in the season, no two of which are exactly alike, is not readily apparent; they are identified provisionally as adult males. These were taken on the following dates: No. 5280, August 4; No. 5283, August 12; No. 5282, August 14 (1940); No. 5177, August 22, 1939; No. 5317, 5318, October 2, 1940; No. 4430, October 4, 1935. The October specimens are evidently examples of retarded moult; each of two had a broken humerus recently healed and the third was emaciated and apparently diseased.

All were in a flightless condition. In No. 5280 and No. 5285 this was due to degeneration of the old flight feathers; in the remainder the flight feathers had been shed and the new feathers were not grown sufficiently to permit flight. In five specimens the rectrices are broken and faded drab to olive buff and in two of these is a single perfect, but not freshly acquired, feather. Another specimen has three feathers of this same type and in another all the old rectrices have been moulted and the new ones, half an inch in length, are concealed by the tail coverts.

In all specimens the ventral surface is pale mouse gray with areas of white where new feathers have appeared; the feathers on flanks are various shades of grey and fawn and in three specimens are several white feathers; the throat is white more or less obscured with a dusky shade; the heads are dusky with faded white cheek patches, in one specimen these and the white throat are confluent; the dorsal surface is mottled fawn, brown, and black, the brown tips of the black feathers on interscapular region forming in one case a definite horizontal pattern, in two others the fresh black secondaries are conspicuous against the brownish background; four have greenish reflections on the occiput, another has definite green and violet areas on cheek and chin.

♂, *Adult.* The adult male wears the familiar plumage, black and white body, white, violet, and green head for nine or possibly 10 months. There is some variation in the time during which individual birds moult but with the greater number it would appear to take place between early July and late August. As stated elsewhere most of them disappear at this time and they do so before any evidence of moult is visible. A specimen taken June 18, 1941, is in full plumage except for slight modification produced by wear. After the flight feathers have been resumed the moult of the body plumage continues for some time and a few adult males may reappear on the nesting grounds before they have attained full plumage. Thus at 103 Mile Lake, August 24, 1937, 22 males in this condition were counted. Early records for fully moulted males are: August 25, 1939; September 15, 1915.

♀, *Juvenals, July–August.* Similar, except for smaller size, to juvenal males. Much browner in general appearance than adult females. The original colours are soon modified. No. 5278, August 14, 1940, has faded to drab and various shades of light brown on the dorsal surface; the tail is cream; on the buff-coloured flanks several new feathers are mouse gray; only the flight feathers retain the original colour and texture.

♀, *First autumn, September–October.* As with the young males there is a gradual moult commencing in the late summer which renews all the plumage except the flight feathers. Thereafter young females more closely resemble adult females, the head and neck are browner, the chest band more fawn than gray, and usually the feathers on dorsal surface are tipped with fawn or gray. There is much individual variation in these indications of juvenility.

♀, *First winter and spring, November–June.* As winter progresses a degeneration in feather structure and loss of colour takes place which at first is most apparent in the tips of the flight feathers and later, on the dorsal surface where the lighter coloured feather edgings become more conspicuous. By May or June the colours have faded to such an extent that second year females may be identified in life with some degree of certainty. Later in the summer the difference in colour between yearling females and older females becomes still more apparent. No. 5008, second year female, August 4, 1938, has faded on the dorsal surface to various shades of brown; cream edgings of feathers on interscapular region form a definite pattern; amongst the old flank feathers, faded almost to white, new gray feathers are conspicuous. In August there is a complete moult, much of the faded body plumage and the faded rectrices being retained until the flight feathers are renewed. On completion of this moult the second year female, then approximately 15 months old, is indistinguishable from the adult female.

♀, *Adult.* The adult female moults at approximately the same time as the second year female. Thereafter the slate, dusky, and white plumage shows little modification until the following summer when some degree of fading takes place. In winter the feathers on the back present a solid coloured surface with bronze reflections in some specimens; the chest band is nearest to neutral gray.

Pattern on greater wing coverts. Young males may have the greater wing coverts above the five or six white middle secondaries unmarked or they may be variously patterned with white. In some this pattern is in the form of an isolated white spot on one, two, or more feathers, in others the terminal third or terminal half of each is white, in others most of one side of the shaft is white or the white is bordered by a dusky tip. In the latter this arrangement forms a narrow bar across the white wing patch (Figs. 7, 8, 9). The same range of variation occurs in the female as in the young male. The pattern appears to be an individual character that is not modified by age (Figs. 10, 11, 12).

Colours of Soft Parts

The colour of the iris in both sexes of all ages is dark brown. In the live adult male in winter and spring the tarsus is alizarine pink; after a bird has been killed and the blood drains from the tarsus it becomes decidedly paler. The following colour notes are from freshly taken specimens.

♂, ♀, *Downy young, approximately two days.* Bill dark neutral gray; nail pale gray; tarsus dark plumbeous.

♀, *Downy young, approximately 12 days.* Bill dark neutral gray; tarsus deep plumbeous.

♂, ♀, *Juvenals, approximately two months.* Upper mandible dark neutral gray; ramus dark Payne's gray; tarsus plumbeous.

♂, *First winter, December.* Sides and basal half of bill dark gull gray; top of bill nearest to olive-gray; interramus purplish gray; tarsus light quaker drab; webs dark quaker drab.

♀, *First winter, December.* Upper mandible neutral gray clouded with deep neutral gray; ramus purplish gray; tarsus light purplish gray.

♂, *Second year, June.* Upper mandible dark neutral gray; ramus dark gull gray; tarsus vinaceous drab inside, dark quaker drab outside.

♀, *Second year, August.* Bill dusky neutral gray; nail paler; tarsus vinaceous slate.

♂, *Eclipse.* Bill darker than in summer; upper mandible neutral gray; ramus dark gull gray; tarsus vinaceous pink.

♂, *Adult, November.* Upper mandible light Payne's gray, slate gray at base; tip of nail black; ramus pale Payne's gray; tarsus, webs, vinaceous fawn.

♀, *Adult, November.* Upper mandible slate colour; ramus light Payne's gray; tarsus pale Varley's gray; webs dark neutral gray.

Food and Feeding Habits

In its feeding habits the buffle-head is not gregarious to the same extent as are scaups and some other diving ducks. Although the population of a feeding ground may number several hundreds it is usual for them to be scattered over the area in small flocks. Many associate in pairs and trios and these seem more vagrant in their movements than are the larger units.

Food is secured almost entirely by diving, the time of submergence ranging between 15 and 35 sec. Sometimes the members of a small flock dive in quick succession so that for a short time all are submerged, or the dives may be so spaced that some birds always are on the surface.

On the sea they show preference for feeding inshore on a rising tide and are conspicuously active when a light surf is breaking close to the beach. Under such conditions some may ride for a short distance on the crest of a wave and as it tumbles into foam avoid the disturbance by a quick dive into calmer water. The sea immediately outside a breaking surf is a profitable feeding ground rich with multitudes of small animals that have been dislodged from their lurking places.

Following the salmon runs on the coast, small numbers of buffle-head frequent the salmon streams and feed on the uncovered salmon eggs. When disturbed they rise quickly and in rapid flight pass down stream to the sea. They fly not far above the surface and well below the tops of the alders that line the banks of most of these streams. They follow each turn of the stream

and seldom short cut over the tree tops as other ducks frequently do under similar circumstances. Buffle-heads also have been seen, in company with other waterfowl, feeding on the eggs of Pacific herring, *Clupea pallasii*.

Food Summaries

The following section summarizes the food eaten by 133 buffle-heads in various localities in the interior and on the coast of British Columbia; the figure following the month indicates the number of specimens examined.

Food of Downy Young

Peace River District: Tupper Creek, June, 2.

Cariboo region: Cummings Lake, June, 1; Lily Pad Lake, July, 1; Tatton Lake, June, 7, July, 2; 105 Mile Lake, June, 1, July, 5; Sheridan Lake, July, 1.

Amphipods. Amphipod fragments constituted 40 to 97% of the total contents of five specimens from 105 Mile Lake.

Insects. Seventeen of the 20 specimens examined had eaten insects, chiefly aquatic nymphs and larvae. In 13 specimens this was the exclusive item; in three others, insects represented 50, 70, and 98% of the total stomach contents. The following occurred the number of times indicated: Corixidae, 6; Chironomidae, larvae, including *Chaoborus* sp., 4, adults, 1; Coleoptera (terrestrial), 3; Coleoptera (aquatic), adults and larvae, including *Dytiscus* sp., *Haliplus leechi*, *Thermonectes* sp., 15; Odonata, dragonfly nymphs, 5, damsel fly nymphs, including *Enallagma* sp., 7; unidentified Diptera, 1; caddis, 2.

Seeds. Thirty seeds of *Polygonum hydropiper* composed 50% of the contents of one stomach; two seeds of *Potamogeton pusillus* constituted a minor item in another.

Miscellaneous vegetation. In three stomachs, unidentified plant material formed 10, 50, and 60% of the contents.

TABLE I

Food of 20 downy young buffle-heads, total percentage volume

Locality	Number of specimens	Amphipods	Odonata	Coleoptera	Corixids	Miscellaneous insects	Miscellaneous vegetation
Tupper Creek	2		31.50	50.00	15.00	3.00	0.50
Lily Pad Lake	1			50.00		50.00	
Tatton Lake	9		40.00	45.67	2.33	12.00	
105 Mile Lake	6	59.50	16.33	1.00	3.00	0.17	20.00
Sheridan Lake	1			40.00		10.00	50.00
Cummings Lake	1			35.00	50.00	15.00	

Food of Adults

Interior Region

Cariboo

Rush Lake, Springhouse, June, 3; 103 Mile Lake, June, 1, October, 2; 105 Mile Lake, August, 6, October, 3; Watson Lake, August, 1; Mirage Lake, August, 1; pond, Lac La Hache, August, 1; Disputed Lake, September, 4; Longbow Lake, September, 8; Lone Butte Lake, September, 2.

Amphipods. Amphipods composed 60 and 70% of the food contents of each of two stomachs from 105 Mile Lake and 60% in another specimen from Disputed Lake.

Aquatic insects. Aquatic insects were present in all but two of the 32 stomachs examined, the most important in volumetric percentage being Odonata. Damsel fly and dragonfly nymphs each occurred in 16, the latter representing 59% of the total volume of food in 12. One bird had eaten over 70 specimens of *Enallagma* sp.; corixid debris was detected in 23 stomachs. In one stomach *Culex* larvae constituted 45% and in another, 5% of the food eaten. Other insects were identified the number of times indicated, viz., May fly nymphs, 3; caddis larvae, 6; dipterous larvae, 2; chironomid larvae, 3.

Coleoptera. Fragments of terrestrial beetles were present in five stomachs and in each represented a minor percentage of the food eaten.

Molluscs. Gastropod fragments were detected in five stomachs; in two composing 50% of the total food content.

Miscellaneous animals. Bryozoan statoblasts were present in six stomachs, in one constituting 89% of the food eaten. One contained a small amount of sponge (Porifera) another, traces of a phyllopod and two others, several leech egg cases.

Algae. A small amount of the filamentous algae, *Spirogyra* and *Zygnema*, was contained in one specimen.

Seeds. Seeds formed, in most cases, a minor item of the total food in a stomach. The most important were *Potamogeton* species and *Myriophyllum;* one contained 50 seeds of *Polygonum* sp. The plants represented were: *Sparganium* sp., *Potamogeton pusillus, P. heterophyllus, P. pectinatus, Zannichellia palustris, Najas flexilis, Carex* sp., *Scirpus* sp., *Rumex* sp., *Polygonum amphibium, Polygonum* sp., *Myriophyllum spicatum.*

Miscellaneous vegetation. Small quantities of unidentified plant material were present in two stomachs.

Okanagan

Swan Lake, September, 3, October, 5, November, 15, December, 4.

Fishes. Fragments of a small sculpin, *Coltus asper*, composed 25% of the food in one December specimen.

Crustaceans. Amphipods constituted from 25 to 60% of the food in four and smaller percentages in nine other stomachs. Both *Gammarus limnaeus* and *Hyalella azteca* were identified; one contained over 165 of the latter. One isopod, sow bug, had been eaten.

Aquatic insects. Insect material was present in 22 of the 26 stomachs examined and in 17 composed 25% or more of the total food content. The following were represented the number of times indicated: May fly nymphs, 6; dragonfly nymphs, 9; damsel fly nymphs, 9; corixids, 21; microcaddis pupae, 6; Coleoptera, adults, *Haliplus leechi*, *Dytiscus* sp., 2; Coleoptera, larvae, including *Dytiscus* sp., 4; chironomid larvae, 10.

Molluscs. A large number of gastropods, *Helisoma trivolvis*, were present in one, several *Lymnaea* sp. in another, and *Planorbis* sp. in three other stomachs.

Miscellaneous animals. Bryozoan statoblasts in five stomachs represented small percentages; in another five leeches averaging 50 mm. composed 50%, and in a third four leeches averaging 55 mm. composed 70% of the total food.

Algae. A small quantity of *Chara* oospores was in one stomach.

Seeds. Seeds of aquatic plants, usually in small amounts, were present in 19 stomachs. One bird had eaten over 200 *Scirpus* seeds, another 61 seeds of *Potamogeton pectinatus*. Seeds of *Zannichellia palustris*, *Ceratophyllum demersum*, and *Potamogeton heterophyllus* also were identified.

Miscellaneous vegetation. Leaves of *Ceratophyllum demersum* were detected in one specimen; small amounts of unidentified plant material occurred in three others.

Okanagan Lake, January, 16, February, 2, March, 1, September, 1, November, 1, December, 2.

Fishes. Bones and other fragments of sculpins, probably *Cottus asper*, occurred in five stomachs and in two of these composed 82% of the total food content.

Crustaceans. Traces of crustacean material were detected in two stomachs.

Aquatic insects. Caddis larvae represented small percentages in 13 stomachs. Ephemeridae (debris), in one case noted as representing about 40 nymphs and in another composing 80% of the total food, were present in seven stomachs. One bird had eaten 275, another 280, chironomid larvae. Corixids were present in three, Dytiscidae, larvae, were minor items in two, and insect debris, not further identified, occurred in nine stomachs.

Molluscs. Molluscs either univalves or bivalves, or both, were present in 14 stomachs with a total percentage volume of 38.52. *Lymnaea vahli* and *Planorbis* sp. were the only identifications.

Miscellaneous animals. Items included under this heading include animal matter, not further identified, that was present in five specimens.

Algae. One stomach contained approximately 6500 *Chara* oospores, another a small amount of *Chara* branches, and a third a small quantity of unidentified algae.

Seeds. Seeds of *Najas flexilis* occurred in nine stomachs; one contained 235 representing 23% of the total food. *Scirpus* seeds were present in 11 and *Potamogeton* seeds in nine. Species of less importance were *Polygonum* sp., *Spartina* sp., *Rubus* sp.

Miscellaneous vegetation. Plant fibre and ground up tubers of *Potamogeton* species constituted 75 and 90% of the contents of two and were identified in two other stomachs. *Najas* fibres occurred in two and small amounts of unidentified plant material in 15 other stomachs.

Vernon Commonage ponds, June, 1, September, 1, October, 5; Pond, Trinity Valley, November, 1.

Crustaceans. One occurrence of an amphipod and *Daphnia* constituted 1% of the food in each of two stomachs.

Aquatic insects. Aquatic insects composed 90.57% of the total food in the stomachs of seven specimens from the Vernon Commonage ponds. One had eaten 280 chironomid larvae, another, a large number of dragonfly nymphs and a third, damsel fly nymphs. *Notonecta* sp. and corixids constituted 90% of the food in the September specimen; the latter composed 50% of the contents in another and were present in four other stomachs.

Miscellaneous animals. Water mites, Hydrachnidae, occurred twice as minor items. The specimens from Trinity Valley had eaten over 1500 bryozoan statoblasts that constituted 75% of the total stomach contents.

Seeds. Seeds of *Potamogeton* sp., *Scirpus* sp., and *Bromus* sp. were minor items in three, 20 seeds of *Potamogeton pectinatus* formed 10% of the food in another stomach.

Miscellaneous vegetation. Plant debris, including *Potamogeton* plant fibre and tubers constituted 15 and 19% of the contents of two and were minor items in three other stomachs.

Coast Region

Fresh Water Habitat

Lower Fraser River, February, 1, November, 1, December, 1; Henderson Lake, November, 1; Quennell Lake, January, 1; Cowichan River, January, 3.

Salmon eggs. The specimen from Henderson Lake contained 25 eggs of sockeye salmon, *Oncorhynchus nerka* as the exclusive item.

Fishes. Rostra and other bones of stickleback, *Gasterosteus aculeatus*, were present in two stomachs from the mainland and in one from Vancouver Island. In one instance this item consisted of approximately 12 and in another of six fishes. Bones of an unidentified fish composed 60% of the contents of a specimen from Cowichan River.

Aquatic insects. Twelve fly larvae (Brachycera) constituted the chief food item in the specimen from Quennell Lake which also contained fragments of corixid adults, chironomid and caddis larvae. One from the lower Fraser River contained debris representing an adult aquatic beetle, corixids, and caddis; in two others, corixids composed 70 and 75% of the contents. At least 200 caddis larvae (Phrygancidae) was the only food in one specimen from Cowichan River.

Seeds. Seeds of *Scirpus* sp. were present in five stomachs, in one case 30, in another 50, had been eaten. Other species represented were *Echinochloa crusgalli*, *Ceratophyllum demersum*, *Potamogeton pectinatus*, *P. foliosus*.

Salt Water Habitat

Kemp Creek, December, 1; Boundary Bay, December, 2; Cowichan Bay, January, 1; Ladysmith Harbour, January, 1; Departure Bay region, December, 5, January, 5.

Fishes. The abraded bones of small marine fishes constituted 60% of the contents of one; a pipe fish, *Syngnathus griseo-lineatus*, 155 mm. in length, was the chief item in a second and bones of a small sculpin occurred in a third specimen.

Crustaceans. Two stomachs contained isopods as the chief item, in one case *Pentidotea* sp., in the other *Exosphaeroma oregonensis*. Amphipod debris was present in five specimens, in one composing 95% of the contents. Fragments of shore crabs, *Hemigrapsus nudus*, were the sole item in two, 98% in another, and represented small percentages in four other stomachs; a small hermit crab, *Pagurus* sp. and several mysids also had been eaten.

Molluscs. Molluscs were the sole item in two, 70 to 90% in six, and small percentages in four other stomachs. One bird had eaten eight tectibranchs that averaged 15 mm. in length and another contained a few barnacle scutes. Species were identified as *Littorina scutulata* Gld., *Colliostoma* sp., *Columbella gausapata* Gld., *C. permodesta* Dall, *Bittium* sp., *Odostomia* sp., *Turbonella* sp., *Thais lamellosa* Gmelin, *Margarites pupilla* Gld., *Alectrion mendicus* Gld., *Lacuna divaricata* (Fab.), *Saxidomus giganteus* (Desh.), *Cardium corbis* Mart., *Paphia staminea* Con. None of the bivalves measured more than 5 mm. in length.

Miscellaneous animals. Jaws of a marine worm *Nereis* sp. was a minor item in a well-filled stomach.

Algae. Marine algae represented five to 10% of the contents of four stomachs.

Seeds. One seed of *Polygonum* sp. in the specimen from Kemp Creek represented the only plant material in this series of specimens.

Summary

Aquatic insects formed the chief food of 13 downy young from five small Cariboo lakes; on another lake amphipods were of first importance. Plant

material was represented in three stomachs, in one forming 50% of the total contents.

Aquatic insects provided the principal food in the summer and autumn diet of 32 adults and full-grown young taken in the Cariboo region and of eight from comparable waters in the Okanagan region. This item composed over 50% of the food eaten by 27 on Swan Lake during the period September 28 to December 5 but was a less important constituent in the food of 30, chiefly winter-taken specimens, from Okanagan Lake. At Swan Lake seeds of aquatic plants were second and amphipods, third in importance. On Okanagan Lake molluscs become more prominent with a total percentage volume of 38.52 and plant food is eaten to a greater extent than elsewhere. Seeds of aquatic plants are represented in small quantities in the majority of specimens from most localities. Chara oospores occurred only once as a major item.

From observation on the coast region it was determined that in some waters salmon eggs are sought for; the one stomach available from a spawning area contained this food exclusively. The food of chief importance on coast lakes and streams is aquatic insects; it is probable that small fishes are taken to a greater extent than the data indicate. Vegetable food is eaten in relatively small amounts. On salt water crustaceans and molluscs are about equal in times of occurrence and total percentage volume. Small fishes are of secondary importance. Herring eggs are eaten in the spring chiefly during the month of March.

TABLE II

FOOD OF BUFFLE-HEAD, TOTAL PERCENTAGE VOLUME

Locality	Number of specimens	Salmon eggs	Fishes	Crustaceans	Insects	Molluscs	Miscellaneous animals	Algae	Seeds	Miscellaneous vegetation
Rush Lake	3				20.00				80.00	
103 Mile Lake	3				46.66	16.67			30.67	6.00
105 Mile Lake	9			14.40	52.60	9.00	11.00	1.12	10.23	1.65
Watson Lake	1				96.00				4.00	
Mirage Lake	1				100.00					
Pond, Lac La Hache	1				100.00					
Disputed Lake	4			15.00	72.00		5.00		8.00	
Longbow Lake	8				94.00		1.00		5.00	
Lone Butte Lake	2				95.50		2.50		2.00	
Swan Lake	27		0.93	9.41	55.44	4.07	5.15	0.11	21.48	3.41
Okanagan Lake	23		7.83	0.09	20.30	38.52	3.91	3.65	4.27	21.43
Commonage Ponds	7			0.29	90.57		0.29		2.71	6.14
Lake, Trinity Valley	1				25.00		75.00			
Lower Fraser River	3		8.00		56.66				35.34	
Henderson Lake	1	100.00								
Quennell Lake	1		10.00		89.00				1.00	
Cowichan River	3		20.00		66.67				13.33	
Kemp Creek	1			99.00					1.00	
Boundary Bay	2		2.50	46.00		48.00		3.50		
Cowichan Bay	1			85.00		15.00				
Ladysmith Harbour	1					100.00				
Departure Bay	10		7.50	50.90		39.50	0.10	2.00		

Economic Status

Like may other species of waterfowl the buffle-head is known to feed upon the eggs of Pacific salmon and Pacific herring. The facts are not in dispute but it seems doubtful if the amount of ova consumed could affect the results of a spawning of either salmon or herring. The number of birds involved is small; the total of eggs available during and after a spawning is vast and of these an uncalculated but probably large percentage becomes a waste product upon which many kinds of birds and fishes feed.

No evidence of buffle-heads eating the fry of salmon or trout has been obtained and their consumption of other small fishes represents a minor item of diet. Stickleback, sculpin, pipe fish, and an unidentified marine fish had been eaten by five of the 15 specimens taken on coast waters and nine of the 118 specimens taken in the interior contained sculpins. Bent (3) cites three authors who refer to small fishes as food items in eastern North America; in one case trout fry is mentioned. Cottam (1) found fish remains representing a total percentage of 3.78 in the total of food eaten by 282 adults from many localities, the most valuable species being one small eel and a sunfish.

The value of the "butterball" as a game bird is less than that of many other ducks, nevertheless it is of some importance. Considered from the standpoint of food it is highly rated as a table bird by the experienced wild fowler in the interior. On the coast where a diet of marine organisms imparts an unpleasant flavour to the flesh it is not so regarded.

Considered from the point of view of "sport" its pursuit attracts chiefly the less experienced hunter. Usually it is the tamest of the ducks and will alight amongst a flock of decoys which may have failed to attract the more desirable species. It has the habit of flying along a lake shore within easy shotgun range and a flock may return to pass a given point several times during a few hours until many of its members have been killed. Nevertheless hunting pressure is not an important reducing factor under normal conditions but in years when the general duck population is below average the pressure is increased to a point that might seriously reduce the population. This was the case in the middle 1930's when drought conditions on the nesting grounds greatly reduced the numbers of those species that the hunter considers most desirable.

The buffle-head possesses in a high degree those qualities of beauty and behaviour that render it an object of affection to many. This is a point that must be considered in an evaluation of the economic status of the species.

From the foregoing it can be concluded that (1) any possible reduction in commercial fish populations is negligible and (2) that the species is otherwise of aesthetic and economic importance.

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References

1. Cottam, A. C. Geo. Wash. Univ., Summaries Doctoral Theses, pp. 66-70. 1937.
2. Cowan, I. McT. British Columbia Provincial Museum, Occasional Papers, No. 1. 1939.
3. Bent, A. C. U.S. Natl. Museum, Bull. 130. 1925.
4. Kelso, J. E. H. Ibis, 2 : 689-723. 1926.
5. Munro, J. A. Condor, 39(4) : 163-173. 1937.
6. Munro, J. A. Trans. Roy. Can. Inst. 22(2) : 259-318. 1939.
7. Taverner, P. A. Condor, 21(2) : 80-86. 1919.
8. Swarth, H. S. Univ. Calif. Pub. Zool. 10(1) : 1-124. 1912.
9. Swarth, H. S. Univ. Calif. Pub. Zool. 24(2) : 125-314. 1922.
10. Swarth, H. S. Univ. Calif. Pub. Zool. 24(3) : 315-394. 1924.
11. Swarth, H. S. Proc. Calif. Acad. Sci. (Ser. 4) 23(2) : 35-58. 1936.

PROTEUS HYDROPHILUS INFECTIONS OF PIKE, TROUT, AND FROGS[1]

By G. B. Reed[2] and G. C. Toner[3]

Abstract

Proteus hydrophilus has been isolated from and shown to be the probable cause of "ulcer disease" of hatchery reared brook trout.

Cultures of *Proteus hydrophilus* isolated from "red sore" diseased pike, "ulcer" diseased trout, "red leg" diseased frogs and from lake water are shown to be indistinguishable by cultural and biochemical reactions.

Proteus hydrophilus infections are shown to be transmissible by water.

In a recent paper by Reed and Toner (3) it was shown that a prevalent disease of adult pike in eastern Ontario waters, known locally as "red sore" is caused by an infection with *Proteus hydrophilus*. It was pointed out that this organism has long been known as the cause of the widespread "red leg" disease of frogs and that the work of Fish (2) associated it with "ulcer disease" of young trout in a New York State hatchery.

1. *Proteus hydrophilus* in Trout

Through the kindness of Mr. W. H. R. Werner of the Ontario Department of Fisheries, the authors recently had an opportunity to examine young brook trout in a small temporary hatchery in eastern Ontario where an epizootic of ulcer disease was in progress, resulting in several hundred deaths per day. The gross lesions in these young trout, 2 to 3 in. in length, apparently fit the descriptions given by Calkin (1) and Fish (2) for ulcer disease as seen in New York State hatcheries. At death the fish had one or more lesions from a few millimetres to a centimetre in diameter, varying from a gray white induration of the skin to deep, necrotic, reddened ulcers extending through the skin into the musculature. These appeared on any part of the body of the fish. The visceral organs, on gross examination, appeared normal except that the kidneys were generally engorged and occasionally necrotic.

The gross appearance in the young trout is very similar to the red sore condition of adult pike except that in the latter species the sores tend to be more superficial with a wider area of infiltration and reddening.

Sixteen trout with conspicuous ulcers, but still living when taken from the tanks, were examined in some detail for parasitic organisms. The culture procedure was that previously followed for pike (3). *P. hydrophilus* was recovered from the necrotic lesions of all 16 fish, from the kidney of six, and from the body fluid of one. All the cultures gave the characteristic reactions of *P. hydrophilus*, Section 5.

[1] *Manuscript received January 19, 1942.*
Contribution from Department of Bacteriology, Queen's University, Kingston, Ont.

[2] *Professor of Bacteriology.*

[3] *Research Assistant.*

The authors have not had an opportunity to test the pathogenicity of the cultures on young trout; however they were tested in goldfish which had previously been found to be easily infected with strains from pike (3). Of the 40 strains, isolated from 16 diseased trout, 10 were selected at random for pathogenicity tests. Twenty-four-hour cultures in broth were injected intraperitoneally into goldfish in 0.1 and 0.01 cc. amounts. The results, summarized in Table I, indicate that all strains tested were pathogenic for goldfish.

TABLE I

PATHOGENICITY IN GOLDFISH OF 10 STRAINS OF *P. hydrophilus* ISOLATED FROM TROUT

Source	Dose of 24-hr. culture	
	0.1 cc.	0.01 cc.
7 strains isolated from skin lesions	Dead in 18 hr. or less	Dead in 48 hr. or less
1 strain isolated from kidney	Dead in 16 hr.	Dead in 30 hr.
2 strains isolated from kidney	Dead in 48 hr.	Not killed.

The fact that *P. hydrophilus* was isolated from the ulcers of all the trout examined and that representative cultures are pathogenic for goldfish makes it highly probable that this is the causal organism of the trout disease. This is in agreement with the conclusion reached by Fish (2).

The origin of the infection in the hatchery was not established. In another section it has been suggested that this organism may be transported by water from a possible reservoir in diseased frogs. But an examination of the water entering this hatchery through a pipe line from a small spring-fed reservoir failed to reveal *P. hydrophilus*.

2. *Proteus hydrophilus* in Lake Water

The evidence appears conclusive that *P. hydrophilus* is the causal agent of "red sore" of pike, "ulcer disease" of trout, and "red leg" of frogs. There is less conclusive evidence that it produces ulcer-like lesions in suckers, bass, perch, and pickerel. It would be of interest to know how it is distributed. To this end the water of some 14 lakes and streams in eastern Ontario was examined for the presence of *P. hydrophilus*. Pike in all of these lakes were known to be infected to a greater or less degree by red sore and frogs in swamps bordering part of the lakes were known to be infected with red leg; the prevalence of red leg in the region suggests that frogs in all the lakes examined were probably infected.

The procedure followed in examining the water consisted in making serial dilutions of the water in sucrose broth. Any that failed to show sucrose fermentation with the largest inoculum, 1 cc. of water, were regarded as free from *P. hydrophilus*. Where fermentation occurred the tube inoculated with the smallest volume of water showing the fermentation was plated on sucrose–brom-thymol-blue agar from which sucrose fermenting colonies were isolated

for detailed identification (3). Cultures isolated in this manner were subjected to the usual biochemical reactions for the identification of *P. hydrophilus* and tested for pathogenicity in goldfish.

Of the 14 lakes and streams examined, four yielded cultures of *P. hydrophilus*. These cultures were indistinguishable from cultures isolated from pike, trout, and frogs (Section 5). All four cultures were pathogenic in goldfish, the infections being fatal. It is possible that a more detailed examination of the water might have indicated a wider distribution. It is, however, significant that the organism was isolated from 4 to 14 samples tested.

These results suggest that the mode of distribution of *P. hydrophilus* to fish is via water, possibly from a reservoir in diseased frogs.

3. Experimental Water-borne Infection

A group of 50 goldfish were exposed to water-borne *P. hydrophilus* infection in the following manner. The goldfish had been for some two months in a cement tank 6 × 3 ft. containing water to a depth of 8 in. Approximately 1 gal. of fresh water per hour was added from a dripping tap. An overflow pipe kept the level constant. There had been no casualties for two months.

Two goldfish infected with *P. hydrophilus* and in a moribund condition were put in the tank. They died within the next few hours and the bodies were not removed until 48 hr. later.

During the next 16 days, six fish died, one on the seventh, ninth, tenth, twelfth, fifteenth, and sixteenth days, after the exposure began. In each instance *P. hydrophilus* was recovered from the kidneys of the dead fish. On the sixteenth day after the beginning of the exposure cultures were made from the slime of the remaining 44 fish. From 21 of the 44, *P. hydrophilus* was recovered and these cultures, injected intraperitoneally into other goldfish, produced fatal infections.

It is evident therefore that contact with two dead fish infected with *P. hydrophilus* in a relatively large tank supplied with flowing water resulted in fatal infections in some 12% of the fish. It is also apparent that the slime of almost 50% of the fish became infected without any noticeable deleterious effects.

4. Optimum Temperature for *Proteus hydrophilus*

If *P. hydrophilus* is distributed by water, as the two previous sections suggest, it is important to know the temperature–growth range of the organism. A series of tubes of sucrose broth were inoculated with six strains of *P. hydrophilus*, two recovered from pike, two from trout, and two recovered from lake water. The results of incubation of the cultures at temperatures from 6° to 40° C. are summarized in Table II. The amount of growth was estimated by comparing the opacity of the broth cultures. It is apparent from the table that growth occurs over a very wide temperature range: from slightly below 6° C. to slightly above 37° C. The optimum growth range, judged by the

TABLE II

TEMPERATURE RANGE FOR GROWTH OF *P. hydrophilus*

P. hydrophilus	6° C.			10° C.			15° C.	20° C.	37° C.		40° C.
	Incubation period, hr.										
	18	40	72	18	40	72	18	18	18	40	72
From Pike 1	−	−	−	+	++	+++	+++	+++	++	+++	−
2	−	+	++	+	++	+++	+++	+++	++	+++	−
From Trout 1	−	−	+	+	++	+++	+++	+++	++	+++	−
2	−	+	+	+	++	+++	+++	+++	++	+++	−
From Water 1	−	+	+	+	++	+++	+++	+++	++	+++	−
2	−	−	+	+	++	+++	+++	+++	++	+++	−

NOTE: (−) = *no growth.*
(+) = *very slight growth.*
(++) = *moderate growth.*
(+++) = *maximum growth.*

amount of growth at the temperatures tested, appears to be between 10° and 15° C. to approximately 30° C.

It is therefore apparent that, if other conditions are favourable, this organism may be expected to grow in lakes from shortly after the ice goes out until shortly before it forms.

5. Comparison of *Proteus hydrophilus* Isolated from Pike, Trout, Frogs, and Lake Water

The possibility of cross infections from pike, trout, or frogs and the possibility of transmission by water depends upon the identity of the infecting organisms in the different hosts. In order to determine the degree of similarity of *P. hydrophilus* from three host species and from water, a comparison has been made of some 17 cultures isolated from red sore of diseased pike taken in different lakes in eastern Ontario; 23 cultures isolated from ulcer disease of young trout in one eastern Ontario hatchery; three cultures isolated from young trout in a Nova Scotia hatchery and kindly sent by Dr. R. H. M'Gonigle of the Atlantic Biological Station; two cultures isolated from red leg of diseased frogs collected in Quebec; four cultures isolated from lake water (Section 3).

Biochemical reactions of the 49 cultures are summarized in Table III. It is apparent that all 49 cultures ferment sucrose, maltose, mannitol, glucose, fructose, trehalose, galactose, and mannose. All 49 fail to ferment lactose, inositol, raffinose, and dulcitol. The fermentation of starch, dextrin, sorbitol, and salicin is variable; about an equal proportion of cultures from the four sources ferment and do not ferment these compounds. All 49 cultures form hydrogen sulphide, form indole, and liquefy gelatine.

TABLE III

BIOCHEMICAL REACTIONS OF 49 CULTURES OF *Proteus hydrophilus* ISOLATED FROM PIKE, TROUT, FROGS, AND WATER. THE FIGURES REPRESENT THE NUMBER OF STRAINS THAT GIVE POSITIVE AND NEGATIVE REACTIONS

	Reaction	17 strains from pike	26 strains from trout	2 strains from frogs	4 strains from water
Lactose	+	0	0	0	0
	−	17	26	2	4
Sucrose	+	17	26	2	4
	−	0	0	0	0
Maltose	+	17	26	2	4
	−	0	0	0	0
Mannitol	+	17	26	2	4
	−	0	0	0	0
Glucose	+	17	26	2	4
	−	0	0	0	0
Levulose	+	17	26	2	4
	−	0	0	0	0
Starch	+	12	20	2	4
	−	5	6	0	0
Dextrin	+	11	18	2	1
	−	6	8	0	3
Inositol	+	0	0	0	0
	−	17	26	2	4
Raffinose	+	0	0	0	0
	−	17	26	2	0
Sorbitol	+	8	17	0	0
	−	9	9	2	4
Trehalose	+	17	26	2	4
	−	0	0	0	0
Galactose	+	17	26	2	4
	−	0	0	0	0
Mannose	+	17	26	2	4
	−	0	0	0	0
Dulcitol	+	0	0	0	0
	−	17	26	2	4
Salicin	+	9	15	2	2
	−	8	11	0	2
H_2S formation	+	17	26	2	4
	−	0	0	0	0
Indole formation	+	17	26	2	4
	−	0	0	0	0
Gelatine liquefaction	+	17	26	2	4
	−	0	0	0	0

The colonial form on nutrient agar shows some variation. The most usual form is an almost entire low convex colony with a very slightly granular surface. On moist agar, most colonies show a tendency to spread in the characteristic amoeboid fashion of the genus. Some strains show the amoeboid tendency to a greater degree than others but no differences of this type could be related to the source of the culture. Very old cultures show a marked tendency to vary from *S* to *R* forms but this again is unrelated to the source. In fluid media all strains form a more or less definite pellicle.

In a previous attempt to relate *P. hydrophilus* isolated from pike and frogs by their antigenic structure (3) it was shown that strains from pike are antigenically so heterogeneous that on this basis no relationship could be shown among cultures from similar hosts. Wenner and Rettger (5) and Taylor (4) found a similar heterogenicity to exist in other species of the genus *Proteus*. Accordingly, no further antigenic examinations have been made.

Acknowledgment

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References

1. CALKINS, G. N. Fourth Ann. Rept. Comm. Fisheries, Games and Forests, New York State. 1899.
2. FISH, F. F. Trans. Am. Fisheries Soc. 64 : 252-258. 1934.
3. REED, G. B. and TONER, G. C. Can. J. Research, D, 19(5) : 139-143. 1941.
4. TAYLOR, J. F. J. Path. Bact. 31(4) : 897-915. 1928.
5. WENNER, J. J. and RETTGER, L. F. J. Bact. 4 : 331-353. 1919.

THE VITAMINS A AND D POTENCY OF THE OILS OBTAINED FROM THE LIVER, INTESTINES, BODY, AND OFFAL OF SHAD, *ALOSA SAPIDISSIMA* WILSON, AND MACKEREL, *SCOMBER SCOMBRUS* L.[1]

By L. I. Pugsley[2], J. T. Kelly[3], W. A. Crandall[4], and C. A. Morrell[5]

Abstract

Data are presented on the percentage of liver and intestines in the fish, percentage of oil in body, liver, intestines, and offal and the vitamins A and D potency, iodine value, and percentage of unsaponifiable matter in these oils of shad and mackerel.

In view of the increased demand for natural resources of vitamins A and D and since no data are available in the literature on the by-products of shad and mackerel, it appeared of interest to make a preliminary survey of the oils obtained from these fish caught from fishing districts in the Maritime Provinces of Canada.

Materials and Methods

Shad caught in the vicinity of Saint John, N.B., and mackerel in the vicinity of Halifax, N.S., were shipped to the Laboratory on ice. There the livers and intestines were removed and weighed and oils were obtained separately from the body, liver, intestines, and offal (heads, livers, and intestines). The vitamin A was determined by the antimony trichloride test using an Evelyn photoelectric colorimeter (3) and vitamin D figures were obtained by biological assay with rats using the line test. Three dosage levels of the samples and standard were employed using 10 rats for each level; the results were calculated according to Bliss and Marks (1, 2).

TABLE I

The weight and percentage of liver and intestines of shad and mackerel

Sample	Date caught	Number of fish	Weight, gm.		Liver in fish, %		Intestines in fish, %	
			Mean	*S.E.*	Mean	*S.E.*	Mean	*S.E.*
Mackerel (1)	2/7/40	42	651	± 34	2.1	± 0.11	3.7	± 0.16
Mackerel (2)	29/11/40	15	781	± 41	1.6	± 0.05	3.6	± 0.09
Shad (1)	20/5/40	14	1752	±143	1.3	± 0.16	3.1	± 0.22
Shad (2)	20/8/40	25	1158	± 26	1.4	± 0.08	4.2	± 0.14

[1] *Manuscript received March 17, 1942.*
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Results

The mean weight, percentage of liver and percentage of intestines in the fish, and the mean percentage of oil in these tissues are presented in Tables I and II, respectively. The vitamin A potency of individual samples and the

TABLE II

THE PERCENTAGE OF OIL IN THE BODY, LIVER, INTESTINES, AND OFFAL OF SHAD AND MACKEREL

Sample	Number of fish	Oil in body, %		Oil in liver, %		Oil in intestines, %		Oil in offal, %
		Mean	*S.E.*	Mean	*S.E.*	Mean	*S.E.*	Mean
Mackerel (1)	16	9.9	±0.9	9.2	±1.2	4.1	±0.4	—
Mackerel (2)	15	19.1	±2.1	16.1	±1.5	13.1	±1.2	16.6*
Shad* (1)	13	20.8	—	15.6	—	14.4	—	—
Shad (2)	12	18.9	±1.5	18.8	±2.8	30.9	±2.8	—

** Duplicate determinations on samples from pooled lots of fish.*

TABLE III

THE VITAMINS A AND D POTENCY OF THE OIL OBTAINED FROM BODY, LIVER, INTESTINES, AND OFFAL OF SHAD AND MACKEREL

Sample	Individual samples			Pooled samples				
	Number of fish	Vitamin A, *L* value per gram*		Number of fish	Vitamin A, *L* value per gram*	Vitamin *D*, International Units	Iodine value	Unsap. matter, %
		Mean	*S.E.*					
Mackerel (1)								
Liver oil	16	87,100	±45,500	26	74,700	1035	151	0.5
Intestinal oil	16	45,100	±21,000	26	57,900	76	160	9.0
Body oil	16	0	—	26	0	20	147	0.3
Mackerel (2)								
Liver oil	15	49,900	± 5900	—	—	—	—	—
Intestinal oil	15	5000	± 770	—	—	—	—	—
Body oil	15	0	—	—	—	—	—	—
Offal oil	—	—	—	30	950	51	140	1.4
Shad (1)								
Liver oil	—	—	—	11	6400	51	153	7.6
Intestinal oil	—	—	—	11	660	20	114	2.9
Body oil	—	—	—	11	0	32	122	0.4
Offal oil	—	—	—	7	160	41	124	5.3
Shad (2)								
Liver oil	12	3600	± 900	13	1500	100	153	3.9
Intestinal oil	12	500	± 170	13	550	21	138	1.0
Body oil	12	0	—	13	0	27	138	0.2

** For the purposes of this report L value per gram is an approximation of the potency in International Units.*

vitamin A and D potency, iodine value, and percentage of unsaponifiable matter in samples of oil obtained from pooled lots of fish are presented in Table III.

Considerable variation in the vitamin potency was found for the individual samples, but the values obtained for mackerel liver oil were within the limits of those usually reported for halibut liver oil, and the shad liver oil approximated the potency of authentic cod liver oil. The body oil of both fish was devoid of vitamin A as shown by the antimony trichloride test. The intestinal oils of mackerel are relatively high in vitamin A but low in vitamin D.

Although the distribution obtained for individual values was not normal, the standard error of the mean is given to indicate the variation.

References

1. Bliss, C. I. and Marks, H. P. Quart. J. Pharm. Pharmacol. 12(1) : 82-110. 1939.
2. Bliss, C. I. and Marks, H. P. Quart. J. Pharm. Pharmacol. 12(2) : 182-205. 1939.
3. Pugsley, L. I. J. Biol. Chem. 128 (Proc. Am. Soc. Biol. Chem. 33) : lxxx. 1939.

CONTINUED STUDIES ON CERCARIAL DERMATITIS AND THE TREMATODE FAMILY SCHISTOSOMATIDAE IN MANITOBA

PART I[1]

By J. A. McLeod[2] and G. E. Little[3]

Abstract

The life cycle of *Pseudobilharziella querquedulae* McLeod, 1937 has been completed experimentally and the relationship of the adult to *Cercaria physella* Talbot, 1936 has been definitely established. The female of the species and the eggs were recovered and are described for the first time. A report is given on the exposure of a number of species of molluscs to the miracidia of this schistosome and notes on the life cycle and adult stage of *Cercaria dermolestes* McLeod, 1940 are included. A case of hypersensitiveness in man to the proteins of schistosome cercariae is reported.

Introduction

The fact that "swimmers' itch" is caused by schistosome cercariae is well known at the present time to the majority of interested people. Each year sees additions made to the already long list of localities reporting outbreaks and also additional records of larvae capable of producing the disease. However, investigations of the biologies of the causative organisms have been singularly unproductive in the past 10 years and in many cases, instead of revealing the details of an organism's life cycle, an extensive investigation frequently has resulted only in the fortuitous discovery of new species of adult schistosomes. The work carried on in Manitoba by the senior author prior to the summer of 1940 is not an exception.

At the outset of the work reported herein, it was known that four species of schistosome cercariae, namely, *Cercaria elvae* (Miller, 1923) Talbot, 1936, *C. stagnicolae* Talbot, 1936, *C. physellae* Talbot, 1936, and *C. dermolestes* McLeod, 1940, were responsible for all the local outbreaks of cercarial dermatitis in man. It was also known that six species of adult schistosomes occur locally in water birds and that, while two of these are of rare occurrence, four species are quite common (3). Three of the more abundant species are found in herring gulls and ring-billed gulls and the fourth in blue-winged teal ducks.

Local ecological evidence of a rather circumstantial nature indicated that there was a possible connection between *C. stagnicolae* and either *Microbilharzia lari* McLeod, 1937 or *Ornithobilharzia aviani* McLeod, 1940, as all three occur in the Clear Lake area. The surveys indicated, however, that while the cercaria in Manitoba was confined to this region, the adult worms were not. Similarly, the ecological evidence indicated a possible connection

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Contribution from the Department of Zoology, University of Manitoba, Winnipeg, Man., with financial aid from the National Research Council of Canada.

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between either *C. elvae* or *C. dermolestes* and *Pseudobilharziella querquedulae* McLeod, 1937.

The senior author had previously reported the finding of adult *P. querquedulae* in immature teal ducks which were, as yet, unable to fly (op. cit.). These birds obviously had been hatched in the vicinity and were collected from a large slough where both *C. elvae* and *C. dermolestes* were very abundant. However, repeated experimental exposure of teal ducks to both species of cercariae failed to produce adult worms. There were no other bodies of water within reach of the infested birds and apparently other species of cercariae were absent from this habitat at the time the collection was made although they may have been present earlier in the season.

Brackett (1) has cited ecological evidence which strongly indicated a connection between *C. physellae* and *P. querquedulae*. The findings set down in this report not only substantiate but prove the accuracy of this assumption. Local studies of snail ecology have not been sufficiently detailed to be of more than superficial value in predicting the course of a schistosome's life cycle. The writers found in 1941 in at least one area under observation that the mature generation of the snail, *Physella gyrina* Say, died off just prior to or at about the same time as young teal made their way to the ponds.

On June 16th, a large lagoon at Delta, Man., was visited but no young teal were hatched as yet. However, mature *Physella gyrina* were abundant in the water and a few were giving off *C. physellae* in large numbers. The same area was visited 10 days later and the reverse condition was found. Freshly hatched teal were plentiful but adult *P. gyrina* had all disappeared and in their place were found many young specimens, none of which was liberating cercariae.

Such observations are inconclusive and only daily observations would determine if there was any overlapping and whether or not *C. physellae* and young ducks occurred simultaneously even for a few hours. Again, the possibility of infection occurring at a later date has not been ruled out and, as pointed out later in this paper, cercariae may emerge as early as 32 days after snail infection. Nothing is known regarding the length of time required for development of the parasite in the bird host but the finding of mature worms in young teal ducks referred to above in early August would lend credence to the theory that infection occurred in the bird during its first few days of life. Brackett's observations provide even stronger evidence in support of this belief.

The prospectus of the work, as in former investigations, centred around the experimental exposure of bird species known to be susceptible to schistosome infection to as many species as possible of local cercariae. In addition it was proposed to recover the eggs from the tissues of the primary host and to attempt to infect local snails with the miracidia.

Materials and Methods

In the experimental exposure of wild birds it is essential, of course, to employ only such specimens as have never come into contact with contaminated water prior to the beginning of the experiment. This necessitates the collecting and artificial incubation of the eggs or the obtaining of young specimens before they have left the nest. The nests of brooding ducks are readily located by adopting the method of having two workers drag a long rope between them over the brush and grass. Artificial incubation of fresh wild duck eggs in the laboratory usually results in a very poor hatch. However, if incubation has been carried on under natural conditions for 10 days or more it usually can be artificially brought to completion quite successfully. The writers' attempts to incubate eggs of the blue-winged teal in the laboratory have been rather unsuccessful and birds for experimental work were kindly supplied by the Delta Duck Station, Delta, Man.

Five young herring gulls were obtained in late June from nests on a barren reef in Grassy Narrows, Lake Winnipeg. While these birds ate readily in captivity when hand fed, care had to be exercised in arranging a diet high in calcium and vitamin D. Birds fed on meat scraps grew normally and were quite fat but in about four weeks they suddenly began to show signs of muscular weakness and lack of bone calcification. A change to a fish diet containing a good deal of bone and the addition of cod liver oil soon corrected the condition.

The locating of the females and eggs of *P. querquedulae* has been attempted by the writers several times in the past but without success. This was mainly due to faulty technique as the females seldom, if ever, occur in the mesenteric veins where the males are most abundant. Later, however, the fine veins under the gut serosa and even those of the submucosa were examined microscopically. This examination revealed moderate numbers of female worms but, because of their minute size, it was possible to recover intact only a small percentage. In the majority, it was necessary to dissect away the surrounding tissue. Ordinary dissecting needles or dental needles are much too coarse for this work but No. 00 insect pins fused into glass handles were found to be fairly satisfactory.

Due to the paucity of eggs in the faeces and the short time required for hatching it is almost impossible to recover the eggs of *P. querquedulae* in ordinary routine faecal examinations. A successful method suggested by Brackett involved splitting the gut and examining the inner surface by transmitted light under fairly high magnification. This technique revealed large numbers of eggs in clusters in the submucosa. They were recovered by dissecting them out individually or by simply scraping off the inner layers of tissue.

Fine, filamentous schistosomes appear to be very sensitive to slight changes in temperature, salt concentration, pH, and other factors, and almost invariably will coil into a tight knot if removed alive from the blood and placed in

any other medium. It was found impossible to relax them by shaking them in a test tube of physiological saline. The writers have found it advantageous to allow the worms to die in the vessels before removal is attempted, providing blood coagulation could be sufficiently retarded. It must be pointed out, however, that post-mortem contraction of the muscles makes recovery of small forms from the vessels of the gut wall very difficult.

The most satisfactory material was obtained from birds that had died during an epidemic of what appeared to be botulism poisoning. It appears that the toxin producing the disease has a paralysing effect on the worms. This suggests that the injection of certain drugs might be very helpful although it has not been tried.

Bouin's picro-formol solution was found to be the most satisfactory fixative for adult schistosomes. Mixtures of Delafield's haematoxylin and alum cochineal gave the best staining results. The proportion of each stain used depends to a great extent on the size of the worm. One part of haematoxylin to three parts of alum cochineal gives good results with large worms but the proportion of haematoxylin should be decreased as the size of the specimen diminishes.

Specimens Examined for Schistosomes

The incidence of snail infestation with schistosome larvae appears to vary enormously from year to year but, like many other organisms in nature, the larvae appear periodically to reach a maximum abundance with cyclic regularity. Even under physical conditions of near stability such as exist at Clear Lake there have been two pronounced outbreaks in the past 10 years, namely in 1933 and 1937, with intervals of comparative scarcity on either side.

While no definite data are available, general observations would lead one to believe that physical factors are not directly responsible for these variations. As regards biotic factors, whereas *Stagnicola emarginata* Sowerby, the snail host, varies considerably in abundance, there appears to be little or no correlation between these variations and the variations in the number of snail infestations. For example, *S. emarginata* was more abundant in Clear Lake in 1940 than during any year since this investigation was begun in 1933, yet infested specimens definitely were more rare. It appears that primary host abundance and the incidence of infestation of this animal species must constitute the variable to a great extent.

General observations and statistical data from former years have not been of great value because of pronounced variations in general conditions. The years prior to 1941 saw a steady and gradual reduction in the precipitation, a lowering of the water table in general, and a decided drop in the water level of marshes, ponds, and lakes. This continued until by early summer in 1940 all small lakes and ponds without sizable inflowing streams were dry and large marshes were very stagnant and much reduced in size. This resulted in a shift of the water bird population from the usual nesting ground to the north-

west and a disappearance of practically all the pond snails except a few of the hardier species. In 1941, heavy snow and rainfall restored these bodies of water to a near normal condition and birds and snails began to appear again. It is readily understood how such extreme conditions disrupt the normal course of events in the life cycles of animals requiring aquatic hosts and having free living aquatic stages.

The commonest representatives of the gastropod fauna of Manitoba had been examined previously for schistosome larvae but it was considered necessary to extend this phase of the work to increase the significance of the results. No complete records of the numbers of each species examined were kept but these ran into hundreds and in most cases thousands.

Species of snail examined	Species of cercaria recovered
Lymnaea stagnalis jugularis (Say)	*Cercaria elvae* (Talbot)
Stagnicola palustris elodes (Say)	*Cercaria dermolestes* (McLeod)
Stagnicola emarginata canadensis (Sowerby)	*Cercaria stagnicolae* (Talbot)
Physella gyrina (Say)	*Cercaria physellae* (Talbot)
Heliosoma trivolvis (Say)	
Gyraulus parvus (Say)	
Pseudosuccinea sp.	
Valvata tricarinata (Say)	
Amnicola sp.	
Amnicola sp.	

The above species comprise more than 90% of the snail population of the prairie lakes and sloughs west of the Precambrian portion of Manitoba. The first seven species are inhabitants of shallow water or occur above the water line but the last three are bottom forms found mainly in deep water and obtainable only by dredging.

Stagnicola emarginata in Manitoba, as in Michigan (McMullen (4)), is the one snail species found in abundance in clear, cold, weed-free water where bathing is most popular. The greatest percentage of cases of swimmers' itch are contracted at Clear Lake which is the habitat of *S. emarginata*. Occasional cases occur in bathers in Lake Winnipeg and Lake Manitoba simply because weedy bays are interspersed with stretches of open shore where the bathing is done. The vegetation is inhabited by *L. stagnalis* and *Stagnicola palustris* and wave motion is sufficient in some cases to carry the escaping cercariae into the water adjacent to the open beaches. People who have the temerity to bathe in sloughs or weedy patches on lake shores almost invariably contract swimmers' itch.

Physella gyrina appears to be only a moderately important factor in connection with dermatitis in Manitoba. This snail species is not rare by any means but rather is quite abundant in a definite type of habitat such as weedy

ponds and lagoons. However, such places are not extensively favoured as bathing places by the public. In addition, specimens of *P. gyrina* examined in the laboratory have shown a rather low incidence of infestation with schistosomes. During the past two summers large numbers have been examined and a relatively small number of infested specimens from the wild state were found. Such infested specimens as occurred were found mainly in the early summer or autumn. This is due to the fact that the mature generation largely dies off about the third week in June and not to any scarcity of adult schistosomes and their hosts.

Young herring gulls, domestic chickens, pigeons, and teal ducks were exposed separately to large numbers of *C. elvae*, *C. stagnicolae*, and *C. dermolestes*. With the exception of the pigeons all were negative when examined at post-mortem five weeks later. The pigeon exposed to *C. elvae* became sick on the 13th day following exposure and died on the 14th day. On examination no schistosomes or eggs were found but the liver was much enlarged, was abnormally pale, and extensively adhered to the body wall in the region of a large abscess on the left lobe. The specimen exposed to *C. dermolestes* was killed and examined on the 44th day and the fine veins of the intestinal wall contained a few mature specimens of a schistosome species.

What appears to be the same species of schistosome had been recovered a couple of weeks previously from the pectoral sandpiper (*Pisobia malanotos*) and the lesser yellow-legs (*Totanus flavipes*). The worms were seen fairly plainly through the wall of the vessels but because of their minute size only fragments were recovered. This material strongly indicates that they are members of the genus *Pseudobilharziella*, but is not adequate for the basis of a specific identification.

Prophylactic and Therapeutic Measures

As pointed out by McMullen (op. cit.) a number of nostrums have found their way on to the market in Michigan both as preventatives and as cures. In Manitoba, few, if any, so-called preventatives are offered for sale but a host of spurious cures are obtainable, none of which, incidentally, is of any value. McMullen suggests as a preventative the rapid drying of the skin with a coarse towel before the adhering water has had time to evaporate. As a palliative lotion he suggests calamine as the most effective and the most easily obtainable.

It has long been considered by the writers within the limits of possibility that the human system might become increasingly allergic to the proteins of schistosome larvae. Only one case has been followed up over a period of years but this one proves the belief to be not without foundation.

The senior author was moderately susceptible to schistosome attacks on first contact with the larvae eight years ago. This disease has been contracted while collecting snails each subsequent summer and the extent of the infestation varied from a few spots to fairly extensive areas. There appeared to be

little or no change in susceptibility but each succeeding attack was accompanied by greater discomfort. During the collecting of *Lymnaea stagnalis* and *Stagnicola palustris* in a lagoon off the Red River near Lockport in 1940, on one occasion an infection consisting of 92 papules was contracted on the right hand and lower six inches of the forearm. The usual itching was experienced for the first 24 hr. but at the end of that time the infected member began to swell and became highly inflamed. This continued for another 24 hr. during which the body temperature rose 1.5° F. and a sensation of general discomfort existed. Dark reddish to purple, torturous streaks appeared on the preaxial side from the wrist to the elbow and there was a marked enlargement and soreness of the supratrochlear and axillary lymph glands.

The infected area was then thoroughly scrubbed with hot water and soap solution and soaked for three ½-hour periods in hot Dakin's solution. Signs of septicemia and inflammation soon subsided and healing took place quickly. However, 10 days later numerous itchy lumps appeared on the skin in various parts of the body. These were reddish and inflamed but did not contain any trace of pus or bacterial infection. An expert dermatologist expressed the opinion that this represented an allergic reaction to the proteins of the foreign organism.

In later operations involving the hand picking of snails, the hand and arm were always given a good coating of solid vaseline. This gave complete protection even though the cercariae were quite abundant in the water.

Chemical control of snails or cercariae was beyond the scope of this present problem.

Descriptions

Pseudobilharziella querquedulae McLeod, 1937 (Fig. 1)

Generic diagnosis: *Pseudobilharziella* Ejsmont, 1929.

Male

Already described, McLeod (2).

Female

Filamentous worms of a creamy white colour, the body somewhat flattened dorsoventrally; almost uniform in width but tapering slightly toward the anterior end. The posterior end of the body is spatulate and in some specimens is expanded giving a suggestion of lobes. The cuticle is smooth or finely tuberculate. The oral sucker is terminal and cone-shaped, the mouth aperture, somewhat ventral in position. The acetabulum is pedunculate and circular in outline with a heavy rim. The oesophagus, a narrow tube, broadens abruptly into an expanded bulb just in front of the acetabulum; from it arise the caecal branches. The caecal branches unite just posterior to the ovary and the common caecum follows a torturous path backwards between the vitellaria to terminate blindly near the posterior end of the body. Its diameter is not uniform but where it comes close to the dorsal or ventral surfaces there is usually an irregular expansion.

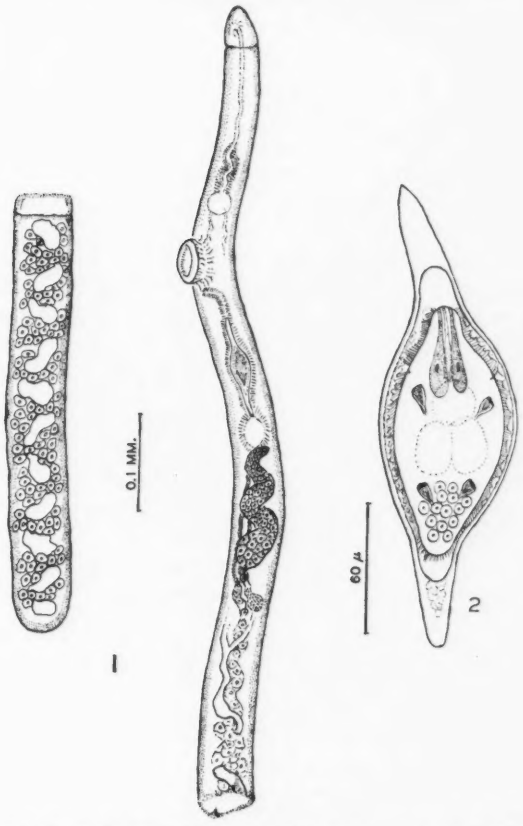


FIG. 1. *Pseudobilharziella querquedulae. Semidiagrammatic sketch of anterior and posterior portions of a stained and mounted female.* ×*150.*

FIG. 2. *Diagram of the egg and miracidium of Pseudobilharziella querquedula.* ×*350.*

The ovary is a stout, spiral, conical structure consisting of about three coils. The basal part of the cone is directed posteriad and the constituent cells are plainly visible. These appear to increase appreciably in size from the anterior to the posterior end. Laurer's canal was not observed to be present, but a well developed sac-like seminal receptacle is located just behind the ovary. Mehlis' gland is at the anterior end of the ovary and the genital pore is just behind the acetabulum. The uterus is moderately straight and contains not more than a single egg. The vitellaria consist of large clearly defined cells with distinct nuclei. These cells are not grouped into follicles but are irregularly dispersed in the region between the ovary and the posterior end. The vitelline cells appear to undergo disintegration as they pass anteriad since they

become granular in appearance, irregular in outline, and the nuclei become less definite as they approach the level of the ovary.

Measurements

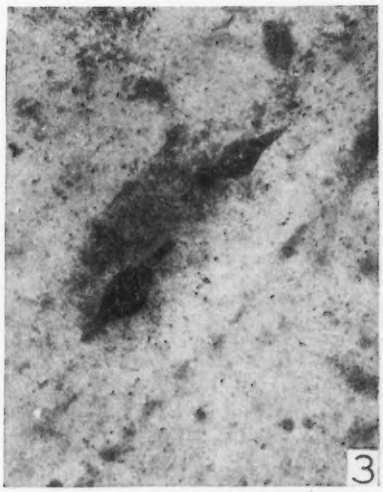
Body length 1.86 mm.; width 60 μ in broadest part and 40 μ at the anterior end; oral sucker 34 μ in maximum diameter; acetabulum located 250 μ from anterior end, 36 μ in diameter; ovary 170 μ behind the acetabulum, overall length, 120 μ (disregarding the total length of the loops), maximum width, 56 μ.

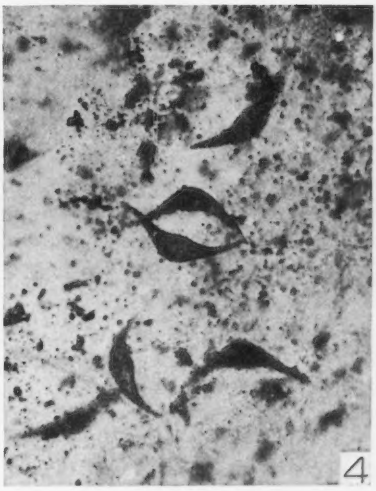
Egg (Figs. 2, 3, and 4)

The average length of 10 normal specimens removed from the intestinal submucosa was 217 μ and the width was 76 μ. The eggs apparently swell after deposition as those measured in the uterus showed an average length of only 140 μ and width of 30 μ. The eggs resemble those of *Bilharziella yokogawi* Oiso, 1927 in that there is a bulbous middle portion and the two ends are drawn out to considerable length and pointed (Fig. 2). The bulbous middle portion comprises approximately one-half the total length of the egg. Frequently the two ends are dissimilar, the anterior being crescent-shaped and sharply pointed while the posterior end is straight, conical, and bluntly pointed. The shell is very thin and fragile excepting the distal portions of the ends which appear to be solid. The large cavity contains a small amount of detritus adhering to the inside of the shell in addition to a well developed and very active miracidium.

The eggs are deposited in the blood vessels of the intestinal muscle layers and submucosa and usually are seen in loosely aggregated groups (Figs. 3 and 4). They occur throughout the length of the gut but are more abundant near the posterior end. Apparently they cause a minimum of damage to the tissue in working their way through to the lumen of the gut as no definite lesions from this cause have been seen. Apart from a slight sloughing of the inner layers of tissue, the gut wall appears to be in a healthy condition. One naturally expects to find lesions when eggs are present in quantity but in this case the absence of lesions cannot be accepted as proof that eggs are not present in the tissue of the intestinal wall.

A great variation in shape, general appearance, and viability of the eggs was observed. The one extreme was represented by a stout egg with a bulbous central portion, a thin, brownish shell, and a well developed miracidium, (Fig. 3). The tissue surrounding this type of egg showed no reaction or only a slight leucocyte infiltration. The other extreme was represented by a slender, crescent-shaped egg with finely attenuated ends and a small central cavity containing only a dark residue (Fig. 4). Each egg of this type was surrounded by a tough fibrous wall and could be removed only with great difficulty. When placed in water they failed to hatch or show any signs of viability. It is considered that these eggs have been embedded in the gut wall for a considerable time. Some bird hosts examined showed a high percentage of eggs of the latter type while in others they were rare or entirely absent.





FIGS. 3 AND 4. *Pseudobilharziella querquedulae. Surface view of a piece of cleared but unstained host intestine showing the eggs in situ. Taken by means of transmitted light using a Wratten green filter.*

FIG. 3. *Photomicrograph of young viable eggs.* ×*90.*

FIG. 4. *Photomicrograph of old inviable eggs.* ×*90.*

Normal eggs hatch in water in from 1 to 20 min. and the miracidium escapes through a longitudinal split in the bulbous part of the shell. The miracidium is of the usual type and is very active. Two pairs of flame cells are present and the nerve tissue is in the form of an indistinct, trilobed mass in the mid-body region.

Life Cycle

Miracidia in moderate numbers were placed in an aquarium containing snails as follows:

Species	Number exposed
Lymnaea stagnalis	10
Stagnicola palustris	11
Stagnicola emarginata	3
Heliosoma trivolvis	3
Physella gyrina	4
Physella sp.	9

These snails were not laboratory reared specimens but had been collected from their natural habitat and kept in the laboratory for a month prior to exposure. They had been checked for emerging cercariae twice before the beginning of the experiment and were rechecked daily thereafter. On the

32nd day following exposure, two specimens of *Physella gyrina* were found to be liberating large numbers of *Cercaria physellae* but all the others were still negative.

During the winter of 1940–41 several specimens of *Physella gyrina* were reared from the egg stage in the laboratory and in the following September four of these were exposed to the miracidia of *P. querquedulae*. Thirty-four days later three of them began liberating large numbers of cercariae. On close examination these proved to be typical specimens of *Cercaria physellae* Talbot, 1936 (5). The emergence of cercariae continued for approximately three months; then the infested snails died.

Discussion

It now remains only to expose teal ducks to *C. physellae* to complete the life cycle stages in the primary host. It has not been possible to do this during the past two years because of the inability to find or produce infested snails at the time when ducks were available. Extreme conditions such as the drying up of ponds and sloughs and the shifting of animal populations were mainly responsible for this. However, in view of the experimental results, both negative and positive, the writers feel they are correct in assuming that *C. physellae* is the larval stage of *P. querquedulae*. It has been demonstrated that *Q. discors* becomes infected with *P. querquedulae* in local waters but repeated exposures of specimens to all known species of local schistosomes cercariae excepting *C. physellae* have always given negative results. In the first exposure of snails to the miracidia it might be argued that the infection was picked up in nature. The results of exposure of laboratory reared specimens eliminates any possible error in this respect.

The examination of bird specimens also shows that *P. querquedulae* occurs fairly commonly in the pin-tail duck (*Dafila acuta*) and the spoon-billed duck (*Spatula clypeata*).

Investigations carried out by the writers so far have shed no light on the life cycle of either *C. elvae* or *C. stagnicolae* or the identity of the adult stages excepting that the cercariae would not infect herring gulls, chickens, pigeons, or teal ducks.

Experimental work did show, however, that *C. dermolestes* is the larval stage of a tiny species of *Pseudobilharziella* occurring naturally in shore birds such as the lesser yellow-legs (*Totanus flavipes*) and the pectoral sandpiper (*Pisobia malanotos*).

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References

1. BRACKETT, S. Am. J. Hyg. D, 32(3) : 85-104. 1940.
2. McLEOD, J. A. J. Parasitol. 23(5) : 456-466. 1937.
3. McLEOD, J. A. Can. J. Research, D, 18(1) : 1-28. 1940.
4. McMULLEN, D. B. State of Michigan, Progress Rept., Div. of Water Itch Contrcl, Stream Control Commission. 1939.
5. TALBOT, S. B. Am. J. Hyg. 23(2) : 372-384. 1936.

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